

# Calculating the metabolizable energy of macronutrients: a critical review of Atwater's results

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*The current values for metabolizable energy of macronutrients were proposed in 1910. Since then, however, efforts to revise these values have been practically absent, creating a crucial need to carry out a critical analysis of the experimental methodology and results that form the basis of these values. Presented here is an exhaustive analysis of Atwater's work on this topic, showing evidence of considerable weaknesses that compromise the validity of his results. These weaknesses include the following: (1) the doubtful representativeness of Atwater's subjects, their activity patterns, and their diets; (2) the extremely short duration of the experiments; (3) the uncertainty about which fecal and urinary excretions contain the residues of each ingested food; (4) the uncertainty about whether or not the required nitrogen balance in individuals was reached during experiments; (5) the numerous experiments carried out without valid preliminary experiments; (6) the imprecision affecting Atwater's experimental measurements; and (7) the numerous assumptions and approximations, along with the lack of information, characterizing Atwater's studies. This review presents specific guidelines for establishing new experimental procedures to estimate more precise and/or more accurate values for the metabolizable energy of macronutrients. The importance of estimating these values in light of their possible dependence on certain nutritional parameters and/or physical activity patterns of individuals is emphasized. The use of more precise values would allow better management of the current overweight and obesity epidemic.*

## INTRODUCTION

Accurate information about metabolizable energy is crucial for the design of public health policies focused on reducing the prevalence and incidence of overweight and obesity. The first systematic efforts to estimate metabolizable energy were made by Rubner<sup>1–3</sup> before 1885. Rubner's work was complemented and vastly improved by Atwater and his coworkers some years

later.<sup>4–27</sup> Incredibly, the values currently used for the metabolizable energy of macronutrients ( $ME_n$ ) are exactly the same as those published in 1910<sup>26</sup>: for carbohydrate (c),  $ME_c = 4$  kcal/g; for fat (f),  $ME_f = 9$  kcal/g; and for protein (p),  $ME_p = 4$  kcal/g.

During the last century, only marginal efforts have been made to corroborate the validity of Atwater's factors in a systematic manner.<sup>28–30</sup> Additionally, a few authors have reported the metabolizable energy for

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specific foods,<sup>31–33</sup> mixed diets,<sup>29,34,36</sup> and certain populations,<sup>35</sup> indicating in some instances important differences (around 20%) between the experimental metabolizable energy and the equivalent energy calculable by Atwater's factors.<sup>29,31–33,35,37</sup>

The values for  $ME_n$  could be expected to show some dependence on one or several of the following factors<sup>28–30,34–36,38–43</sup>: (1) the amount and/or type of fiber ingested; (2) the amount of water ingested; (3) the percentage of caloric excess or deficiency in the diet; (4) the relative amount of each macronutrient ingested daily; (5) the variety of ingested foods; (6) the extent of chewing; (7) the average number of meals per day and their relative caloric distribution; (8) the extent to which foods are cooked; (9) the presence and amount of alcohol in the diet; (10) the daily physical activity pattern of individuals; (11) the sex, age, and life stage of individuals (eg, infancy, adolescence, adulthood, late adulthood/elderly, etc); (12) the presence and stage of certain diseases in individuals; and (13) the level of stress experienced by individuals. Atwater<sup>23</sup> considered some of these factors, but the multiple errors and/or the lack of precision in his experiments render his data insufficient to reach definitive conclusions.

Despite the above-mentioned shortcomings, the  $ME_n$  values proposed by Atwater are still applied almost universally. Given this, a critical review of Atwater's results is presented here. Scrutiny of the manner in which Atwater obtained his  $ME_n$  values reveals undeniable evidence of numerical errors, currently unacceptable approximations, experimental inaccuracies, and conceptually erroneous assumptions, whose elimination would allow, a priori, the calculation of more accurate  $ME_n$  values. Estimating the metabolizable energy of foods more precisely will not only strengthen the scientific foundation of nutritional science, it will also improve the professional practice that promotes refinement of the population's eating behaviors.

## ATWATER'S CONTRIBUTIONS

Atwater's research on the metabolism of matter and energy began formally in 1892; his experiments with humans, however, commenced during the winter of 1895–1896, when he began using his respiration calorimeter.<sup>6,10</sup>

The extensive material published by Atwater and his coworkers<sup>4–27</sup> was presented in a fragmented, disordered, often repetitive manner and was distributed in more than 2000 pages published between 1891 and 1910. Those pages include not only several calculation and typographical errors (which have been corrected for this review) but also numerous corrections or recalculations of data; in fact, in most experiments, up to 3

different values for the same experimental parameter were reported by Atwater in different publications. Moreover, practically all of Atwater's works contain one or more relevant errors that will be discussed throughout this review. Nevertheless, it is important to recognize Atwater's honesty, since he explicitly reported the arbitrary assumptions and/or poorly supported decisions considered in several experiments. Despite the limitations of his research, Atwater has been recognized as one of the founding figures of nutritional science in America.<sup>46</sup> The relevance of his contributions is undeniable, and his work has been recognized worldwide over the last century.<sup>44–46</sup>

## Metabolizable energy

A suitable starting point to sum up Atwater's contributions is his definition of the “fuel value” of food, which, in general terms, is very similar to the concept of metabolizable energy.<sup>37</sup> In practice, both terms are commonly considered to be equivalent. Atwater defined his fuel value as “. . . the energy of the material of food that is capable of oxidation in the human body.”<sup>12</sup>

For fats, as well as for carbohydrates, the metabolizable energy is the total energy ingested by means of a specific nutrient (ie, the amount of kilocalories produced by the combustion of each gram of fat or carbohydrate ingested), minus the heat of combustion of the unoxidized (or partially oxidized) material expelled in what Atwater termed “the corresponding feces.” In the initial version of this definition, the corresponding feces consisted of only the residual materials produced by each gram of the ingested fat or carbohydrate, but, after a more in-depth analysis,<sup>12,13,21</sup> Atwater modified this definition to include in this fraction the residues of digestive juices, the fragments of intestinal epithelium, and other minor compounds or residues (collectively described as “metabolic products”) that are usually excreted in feces together with the unoxidized (or partially oxidized) nutrients. Therefore, the  $ME_n$  values can be calculated by means of the following equation:

$$ME_n = HC_n - F_n \quad [1]$$

where  $n$  denotes  $c$  (carbohydrates) or  $f$  (fats),  $ME_n$  is the metabolizable energy by mass unit of the corresponding nutrient (expressed in kilocalories per gram of the nutrient contained in the ingested food),  $HC_n$  is the heat of combustion by mass unit of the corresponding non-nitrogenous nutrient (expressed in kilocalories per gram of non-nitrogenous nutrient, as it is contained in food), and  $F_n$  is the heat of combustion (measured in kilocalories) of the dry fecal residue produced by each

gram of non-nitrogenous nutrient ingested in food (including the corresponding metabolic products).

It is well known that proteins ( $p$ ) cannot be completely oxidized by the human body, as protein metabolism produces urea, uric acid, creatinine, and other minor nitrogenous compounds that are usually excreted in urine. Therefore, the  $ME_p$  value corresponds to the heat of combustion of each gram of protein ingested, minus the heat of combustion of the residues of each gram of ingested protein that are excreted in the feces or in the urine. Thus, the  $ME_p$  value can be calculated by using a variation of equation [1], presented here as equation [2], which includes an extra term to consider the nitrogenous compounds excreted in urine.

$$ME_p = HC_p - F_p - U_p \quad [2]$$

In equation [2],  $ME_p$  is the metabolizable energy of the protein (expressed in kilocalories per gram of protein contained in the ingested food), and  $U_p$  is the heat of combustion (measured in kilocalories) of the dry urinary residue produced by each gram of protein ingested in food.

Because human excretions consist mostly of feces and urine, Atwater explicitly considered only feces and urine in the original proposal of equations [1] and [2].<sup>12</sup> Nevertheless, for his “work experiments,” he also considered perspiration,<sup>11,19–21,23</sup> finding that the energy loss from it was comparatively very low (representing around 2% of the total losses). Losses through respiratory products and intestinal gases were not considered by Atwater.

To obtain the metabolizable energy of a given amount of a certain macronutrient, the  $ME_n$  value obtained with equation [1] or [2] must be multiplied by the corresponding  $G_n$  value, with  $G_n$  being the mass of the respective macronutrient expressed in grams. In addition, since metabolizable energy is additive, the metabolizable energy of a certain amount of food containing more than one macronutrient can be calculated by summing the metabolizable energy of the mass of each macronutrient included in the specific food.

On the basis of the above, relevant information will be presented about the procedure Atwater used to obtain the following parameters required to estimate the metabolizable energy of a given amount of food: (1) the heat of combustion of certain mass of a given macronutrient [=  $(G_n)(HC_n)$ , expressed in kilocalories]; (2) the availability of each macronutrient ( $A_n$ ) [ $A_n = (HC_n - F_n)/HC_n$ ]; and (3) the energy of the nitrogenous compounds excreted in urine [=  $(G_p)(U_p)$ , expressed in kilocalories].

## HEAT OF COMBUSTION OF THE INGESTED MACRONUTRIENTS

### Protein mass and heat of combustion of proteins

Atwater<sup>13</sup> applied the term *protein* (named *protein fraction* here) to all nitrogenous nutrients present in food, with the exception of nitrogenous fats. He subdivided the protein fraction into proteids (eg, albumen in meat and egg, casein in milk, myosin in meat, gluten in wheat, and chondrogen, gelatin, etc.) and nonproteids (eg, creatine, creatinine, other extractives of meat, amides in vegetable foods, etc).

Around 1900, it was universal practice to determine the nitrogen content of a food sample and to estimate the protein content by multiplying the nitrogen content by a pre-established factor<sup>13</sup>; such practice is still common.<sup>47</sup> Atwater considered the universal factor to be 6.25 g of protein per gram of nitrogen; it is equivalent to consider the average nitrogen content of the protein fraction of any food (or mixed diet) to be 16%. However, such approximation involves the errors described below.

(1) Atwater affirmed that the average nitrogen content of protein fractions is approximately 16%, but only for protein fractions obtained from animal sources.<sup>13</sup> Nevertheless, in a previous study,<sup>4</sup> he measured the nitrogen content of numerous fishes and aquatic invertebrates, finding values between 14.8% and 17.3%. Moreover, for vegetables, he reported nitrogen contents of 16.7% (maize, oats, buckwheat, rice, and their manufactured products) and 17.7% (vegetables);<sup>13</sup> however, current data shows values as high as 19.4% (cucumber) and 30.5% (spinach).<sup>48</sup> As a consequence of this systematic error, the protein content in a given sample is usually overestimated; this was explicitly recognized by Atwater.<sup>13</sup> The inherent error in the above assumption can be exemplified as follows: when the actual nitrogen content of the protein fraction in a given food is 17.5%, the protein content of the food sample is overestimated by approximately 10%.

(2) Usually, foods contain a certain amount of nonproteids, which, taken collectively, have a higher average nitrogen content and a lower heat of combustion than proteids. In addition, for animal-source foods, Atwater affirmed that the representative nonproteid is creatine (which has a nitrogen content of 32% and a heat of combustion of 4.27 kcal/g<sup>13,49</sup>), whereas for vegetable-source foods, it is asparagine<sup>13</sup> (whose nitrogen content is 21% and heat of combustion is 3.45 kcal/g<sup>13</sup>). Thus, the presence of nonproteids produces an additional systematic error when estimating the heat of combustion of the protein fraction ( $HC_p$ ) present in each food.

(3) Despite the limited information available at the time of his studies, Atwater affirmed that the relative content of nonproteids in a given sample strongly depends on the type of food, reporting values of  $\leq 4\%$  for meats, cereals, and legumes, 30% for fruits, and 40% for vegetables.<sup>13</sup> Therefore, the effect of the presence of nonproteids could become considerable when the factor 6.25 is used as a universal factor to estimate the mass of the protein fraction ( $G_p$ ) present in a given sample.

To estimate the  $HC_p$  value, Atwater used the following values (expressed in kilocalories per gram),<sup>13</sup> without providing any information about their bibliographic sources: beef and veal (fat-free muscle), 5.65; mutton (fat-free muscle), 5.60; beef (fat-free muscle, extractives removed), 5.73; egg albumin, 5.71; egg (yolk protein), 5.84; vitellin, 5.76; milk casein, 5.63–5.86; milk protein, 5.67; wheat gluten, 5.95; legumin, 5.79; plant fibrin, 5.89; glutenin, 5.83; and gliadin, 5.92.

Using these values and a set of more or less arbitrary and imprecise assumptions, Atwater estimated the following heat of combustion values (expressed in kilocalories per gram) for proteids present in several food groups<sup>13</sup>: animal-source foods, 5.7; cereals, 5.9; and dried legumes, vegetables, and fruits, 5.8. Thus, he used this last set of values and the corresponding relative contents of nonproteids to estimate the following roughly rounded values (expressed in kilocalories per gram), which were considered representative for each food group: meats, 5.65; eggs, 5.75; dairy products, 5.65; cereals, 5.80; legumes, 5.70; vegetables, 5.00; and fruits, 5.20.

An additional arbitrary average was proposed by Atwater<sup>13</sup> when he regrouped meats, eggs, and dairy products as “animal foods,” assigning them the following value:  $HC_p^{[animal]} = 5.65$  kcal/g. It is evident that, except for egg-free diets, the value of 5.65 kcal/g is slightly underestimated. In a similar way, Atwater regrouped cereals, legumes, vegetables, and fruits as “vegetable foods” and arbitrarily defined  $HC_p^{[vegetable]} = 5.65$  kcal/g. Considering that he proposed values of 5.2 kcal/g and 5.0 kcal/g, respectively, for the heat of combustion of fruits and vegetables, the use of a generalized value of 5.65 kcal/g would result in an up to 12% overestimation for these groups, which can become important when applied to diets high in fruits and vegetables.

Despite the above-described approximations, Atwater’s final proposed value for  $HC_p$ , recommended for any type of food, was 5.65 kcal/g.

### Fat mass and heat of combustion of fats

Atwater recognized that, especially in meat samples, the ether extraction of fatty components could be incomplete, causing underestimation of the fat content of the

meat.<sup>13</sup> To estimate the average heat of combustion of fat ( $HC_f$ ), Atwater used the following values (expressed in kilocalories per gram)<sup>13</sup>: beef and pork fat, 9.50; ether extract of beef and pork, 9.24 and 9.13; mutton (fat and ether extract), 9.51 and 9.32; lard, 9.59; butter fat, 9.27 (9.179<sup>1</sup>); wheat (oil and ether extract), 9.36 and 9.07; rye (oil and ether extract), 9.32 and 9.20; maize oil, 9.28; ether extract of oats, 8.93; olive oil, 9.47 (9.384<sup>1</sup>); ether extract of barley and coconut oil, 9.07; and nut oil (except coconut), 9.49. Specific references for these values were not reported, although several values coincide with those reported by Wiley and Bigelow.<sup>50</sup>

Afterward, Atwater proceeded to use an unreported procedure to average such values and propose the following representative values for  $HC_f$ : (1) for animal-source fats,  $HC_f^{[animal]} = 9.4$  kcal/g; and (2) for vegetable-source fats,  $HC_f^{[vegetable]} = 9.3$  kcal/g.

Considering that, in the case of meats, the  $G_f$  value used to calculate the total heat of combustion of a given food sample is obtained experimentally from an ether extraction, it would be recommendable, to be consistent, to consider as representative values those heat of combustion values measured from the respective ether extracts. Thus, in the case of animal-source fats, it is clear that, with the exception of lard (practically absent in the “healthy eating pattern”<sup>51</sup>), the remaining values given are lower than 9.4 kcal/g, and their average is 2% lower than 9.4 kcal/g. An equivalent analysis shows that all the reported heat of combustion values for ether extracts of vegetables are lower than 9.3 kcal/g; in fact, the average of these values is 2.5% lower than 9.3 kcal/g. In the case of oils, current information about the worldwide consumption of oils for edible purposes shows that, today, the oils studied by Atwater are not widely consumed.<sup>52</sup> Therefore, these oils could a priori be considered as not representative of the current global reality.

Finally, based on the results published in 185 American dietary studies (the references were not mentioned, but it is probable that he included his own dietary studies<sup>7–9,15–18,22</sup>), Atwater claimed that roughly 92% of the fat eaten by Americans came from animal sources,<sup>13</sup> leading him to recommend the use of  $HC_f = 9.4$  kcal/g for any fat. Current recommendations for a healthy eating pattern state that fat intake from animal sources should be less than one-third of all fat ingested,<sup>51</sup> which implies a considerable difference from Atwater’s claim.

### Carbohydrate mass and heat of combustion of carbohydrates

As a consequence of both the “difference method,”<sup>47</sup> usually used to quantify carbohydrates, and the overestimation of the protein content (see discussion in the *Protein mass and heat of combustion of proteins* section)



of a sample, the carbohydrate content is usually underestimated. In fact, although Atwater recognized that meats and fish contain small amounts of carbohydrates,<sup>13</sup> the estimation of the mass of carbohydrates ( $G_c$ ) in these types of samples by strict application of the difference method usually produced a negative carbohydrate content, and therefore he arbitrarily considered such content as zero; such practice is still common today.<sup>53</sup> Nevertheless, Atwater's main error about this topic was that he considered fiber to be a completely metabolizable carbohydrate. This inaccuracy can become particularly important when considering healthy diets (eg, those in which the ingested fiber represents more than 10% of the mass of ingested carbohydrates).

Atwater assumed that most of the animal-source carbohydrates ingested by Americans came from milk and dairy products<sup>13</sup>; however, this assumption cannot be justified a priori as being representative of the current situation.<sup>54</sup> Besides, although he explicitly recognized that there were different figures for the heat of combustion of lactose,<sup>13</sup> he considered, without any additional explanation, 3.86 kcal/g as a representative value for the heat of combustion by mass unit of carbohydrates ( $HC_c^{[dairy\ products]}$ ) present in dairy products; alternative values for the heat of combustion of lactose are 3.877 kcal/g<sup>1</sup> and 3.94 kcal/g.<sup>37</sup> On the basis of the above, he proposed a final rounded figure, affirming that, for any animal-source food,  $HC_c^{[animal]} = 3.9$  kcal/g.

To estimate the heat of combustion of carbohydrates obtained from a vegetal source, Atwater used the following values (expressed in kilocalories per gram):<sup>13</sup> starch and cellulose, 4.20 (alternative values for starch, 4.12<sup>1</sup> and 4.18<sup>37,55</sup>); cane sugar, 3.96; dextrin, 4.11; pentoses, 3.72–4.38; dextrose, 3.75; levulose, 3.76; and sucrose, 3.96 (3.959,<sup>1</sup> 3.94,<sup>37</sup> 3.91<sup>55</sup>). Although he did not report specific references, some of his values coincide with those reported by Wiley and Bigelow.<sup>50</sup> Finally, Atwater proposed that  $HC_c^{[vegetable]} \approx 4.2$  kcal/g.<sup>13</sup>

To demonstrate the error of applying the value of 4.2 kcal/g to fruits, consider the following relative amounts of the different carbohydrates commonly contained in fruits, along with the respective heats of combustion: fructose, 0.4%–23.7%<sup>56</sup> and 3.73–3.75 kcal/g<sup>37,50</sup>; glucose, 0.5%–32%<sup>56</sup> and 3.692–3.72 kcal/g<sup>1,37</sup>; sucrose: 0.0–8.2%<sup>56</sup> and 3.94–3.96 kcal/g<sup>1,13,37,50,55</sup>; starch and fiber, 0.3%–2.5%<sup>56</sup> and 4.12–4.2 kcal/g (for starch)<sup>1,13,37,50,55</sup> or 4.2 kcal/g (for fiber).<sup>13,50</sup> Qualitatively speaking, since most of the components have combustion values below 4.2 kcal/g, the overestimation of the heat of combustion of the carbohydrates contained in fruits is evident. As an example, consider a hypothetical fruit containing 10% fructose, 15% glucose, 5% sucrose, and 2% starch and fiber (note that these values are within the previously mentioned intervals); in this hypothetical case, the use of

the generalized value results in an approximately 11% overestimation of the heat of combustion value (the actual value is 3.79 kcal/g).

Additionally, since the added sugar intake has increased greatly in recent years,<sup>57,58</sup> the overestimation that will result from using 4.2 kcal/g (the proposed universal value) instead of 3.95 kcal/g (averaged value of the available data<sup>1,13,37,50,55</sup>) could become important (approximately 6%).

Finally, taking into account the previously mentioned dietary studies, Atwater claimed that around 95% of carbohydrates eaten by Americans came from a vegetal source, and, without any additional quantitative support, he proposed that  $HC_c = 4.1$  kcal/g for the carbohydrates present in all types of foods.

A critical analysis of the section *Heat of Combustion of the Ingested Macronutrients* shows that application of the generalized  $HC_n$  values could objectively result in a high level of inaccuracy. An additional factor is the improvement in the calorimetric equipment and techniques over the last century.<sup>59,60</sup> Then, extrapolating from this improvement, one would expect that the current calorimetric data are more accurate and precise than those obtained by Atwater. Thus, in hindsight, both aspects would justify the calculation of new values for at least some of the foods studied by Atwater as well as for others on the wide spectra of modern foods. Considering the above, numerous calorimetric determinations of several foods and their fractions have been performed,<sup>61</sup> and an original paper in which an improved set of  $HC_n$  values will be proposed is being prepared for publication.

### Availability of the ingested macronutrients

Atwater decided to estimate the available energy by mass unit of each of the 3 macronutrients, that is, the  $HC_n - F_n$  values, as a fraction of the heat of combustion by mass unit of the corresponding macronutrient (the respective  $HC_n$  value), defining a set of 3 coefficients, named here as “availability coefficients” ( $A_n$ ), which are defined mathematically as follows:

$$A_n = \frac{(HC_n - F_n)}{HC_n} \quad [3]$$

Despite the definition of  $A_n$  presented in equation [3], in all their work, Atwater and coworkers<sup>5,11,19–21,23,24,27</sup> calculated the  $A_n$  coefficient by determining the mass of each nutrient in the food ingested and in the feces, using the following formula:

$$A_n = \frac{(G_n - M_n^{(f)})}{G_n} \quad [4]$$

where  $M_n^{(f)}$  is the grams of the respective macronutrient expelled in feces that are produced for each gram of such macronutrient ingested.

Nevertheless, complete equivalence between equations [3] and [4] is only possible when the following conditions are met: (1) the macronutrient expelled in feces is not chemically altered by the digestion process, remaining completely undigested at the end of the digestive apparatus; and (2) the expelled fraction does not contain metabolic products. Usually, neither of these two conditions is strictly fulfilled. Therefore, the use of equation [4] instead of equation [3] involves a very important conceptual error that must be avoided in the future. In the hypothetical case that these two conditions have been fulfilled, the heat of combustion by mass unit of the macronutrient ingested is the same as the heat of combustion by mass unit of the expelled fraction, which ensures the validity of the following equation:

$$F_n = \frac{(HC_n)(M_n^{(f)})}{G_n} \quad [5]$$

In equation [5], the numerator represents the heat of combustion of the expelled fraction (expressed in kilocalories); note that the  $F_n$  value is expressed in kilocalories per gram of the nutrient ingested, just as it was defined for equation [1]. When  $F_n$  in equation [3] is calculated as shown in equation [5], equation [4] can be obtained algebraically. Thus, to define the  $A_c$  and  $A_f$  parameters (of equation [3]) in equation [1], equation [1] can be modified as follows:

$$ME_n = HC_n(A_n) \quad [1 \text{ modified}]$$

In the case of protein ( $p$ ), by substituting the  $A_p$  definition (of equation [3]) in equation [2], equation [2] can be modified as follows:

$$ME_p = HC_p(A_p) - U_p \quad [2 \text{ modified}]$$

## Availability values

In 1900, Atwater published a preliminary set of availability ( $A_n$ ) parameters, considering them valid for some types of foods (animal foods, cereals, legumes, sugars and starches, fruits, vegetables, and vegetable foods).<sup>13</sup> The detailed procedure used to obtain such values was not mentioned explicitly, but the author admitted that the method was imprecise, affirming the following: “There is more or less guess work in the

method of estimating the coefficients of availability.” Further evidence of his objectivity and honesty is provided by another statement: “We do not assume that the coefficients represent the actual availability of the nutrients of the different kinds of food materials under all circumstances or in all of the food materials of any given class.”

To estimate such parameters, an unreported number of digestion experiments using “single food materials” or very simple mixed diet foods were considered, in addition to almost 100 digestion experiments performed in 13 men in the United States on complex mixed diets.<sup>5,11,62,63</sup> From the results of these experiments, Atwater recommended the following parameters for animal foods<sup>13</sup>:  $A_p = 0.97$ ,  $A_f = 0.95$ , and  $A_c = 0.98$ . However, for the different types of vegetable foods, he reported that parameter  $A_p$  ranged from 0.78 to 0.92, and parameter  $A_c$  ranged between 0.90 and 0.98; he reported  $A_f$  as a unique value of 0.90.<sup>13</sup> Finally, generalizing using a method that was not reported (and, at first sight, arbitrarily determined), he proposed the following preliminary values<sup>13</sup>:  $A_p = 0.92$ ,  $A_f = 0.95$ , and  $A_c = 0.97$ , asserting that these values can be applied to any type of food.

Nevertheless, after 1900, Atwater and coworkers carried out many other digestion experiments,<sup>19–21,23,24,27</sup> reporting in total 79 suitable sets of the 3 parameters for  $A_n$ . For proteins, the  $A_p$  values were between 0.818 and 0.968, averaging 0.912. For fats, the value of  $A_f$  ranged from 0.883 to 0.983, with an average of 0.951. Finally, for carbohydrates, the  $A_c$  value ranged between 0.937 and 0.989, with the average being 0.976. When these average values are compared with Atwater’s preliminary values, the respective differences can be considered negligible. Nevertheless, it is important to point out the high variability of the reported  $A_n$  values, which, in the case of protein, is approximately  $\pm 10\%$ . Such variability can be used as support to claim, at least hypothetically, that the  $A_n$  parameters depend on the specific eating pattern and/or the physical activity level of the individual. Since the current-day eating habits differ considerably from those considered during Atwater’s experiments, the actual variability of the  $A_n$  values could be expected to be much higher than that reported by Atwater. In addition, the respective average values of the actual measurements for each  $A_n$  parameter would likely be considerably different from those reported by Atwater.

## Loss of protein in urine

In 1900, Atwater affirmed for the first time that, by considering 46 determinations of the heat of combustion of urinary solids and their respective nitrogen contents

(he clearly recognized that not all experiments were conducted by him), the average energy lost in urine was 7.9 kilocalories per gram of nitrogen in urine.<sup>13</sup> In addition, he assumed that: "...one gram of nitrogen in urine represents the breaking down or catabolism of 6.25 g of available protein of food or of body protein," recognizing that "...this assumption is slightly inaccurate due to the presence of some proteids in food, such as those of wheat and rye, and more specially of the non-proteids containing more than 16% of nitrogen" (see additional comments in the section *Protein mass and heat of combustion of proteins*).<sup>13</sup> Thus, Atwater's final proposal for  $U_p$  (loss of protein derivatives in urine) was as follows:  $U_p = 7.9/6.25 = 1.25$  kilocalories per gram of protein in urine.

After 1900, Atwater conducted many other digestion and metabolism experiments to measure  $U_p$  values<sup>19–21,23,24,27</sup>; in total, he carried out 99 experiments. For the digestion experiments (29 experiments), only one average  $U_p$  value for each experiment could be calculated, whereas in the metabolism experiments (70 experiments over 197.5 days), a daily value could usually be calculated. In some such experiments, however, certain experiment-related problems prevented daily values from being obtained, instead allowing only the corresponding average  $U_p$  values to be calculated. Thus, only 214 suitable  $U_p$  values are available, ranging between 5.2 and 12.8 kcal per gram of nitrogen and averaging 8.2 kcal per gram of nitrogen. In a more recent study, urine samples from 9 individuals were studied, with  $U_p$  values ranging from 4.96 to 19.05 kcal per gram of nitrogen being reported.<sup>29</sup>

At first glance, 8.2 kcal per gram of nitrogen is very similar to the value proposed by Atwater in 1900 (7.9 kcal per gram of nitrogen<sup>13</sup>). However, an analysis of the 214  $U_p$  values calculated using Atwater's data (and the values more recently reported<sup>29</sup>) establishes, at least as a reasonable hypothesis, that there is an important dependence of this parameter on the specific eating pattern and/or physical activity level of the individual.

To contribute to this topic, a revised set of measurements have been developed with the aim of proposing an improved  $U_p$  value<sup>64</sup> that is significantly different from the equivalent Atwater's value. This information is expected to be published in the near future.

### Universal values for metabolizable energies

By using equations [1 modified] and [2 modified] and the values for  $HC_m$ ,  $A_m$ , and  $U_p$  that he reported in 1900, Atwater presented the following rounded estimated values for metabolizable energies<sup>13</sup>:

For proteins:  $ME_p = HC_p(A_p) - U_p = 5.65 (0.92) - 1.25 = 4.0$  kcal per gram of protein  
 For fats:  $ME_f = HC_f(A_f) = 9.4 (0.95) = 8.9$  kcal per gram of fat  
 For carbohydrates:  $ME_c = HC_c(A_c) = 4.1 (0.97) = 4.0$  kcal per gram of carbohydrate

Afterward, in 1910, Atwater published a final rounded figure without providing any supporting evidence,<sup>26</sup> proposing the  $ME_n$  values as 4 kcal/g, 9 kcal/g, and 4 kcal/g for proteins, fats, and carbohydrates, respectively.

On the basis of the information provided above, it is clear that the numerous data obtained by Atwater after 1900 were not considered in his proposal of the final  $ME_n$  values. These final values are now used worldwide. Currently, they are used not only to estimate the metabolizable energy of mixed diets (as proposed originally) but also to assess the metabolizable energy of individual foods, which, in light of the evidence presented in this review, can result in considerable errors. Since the effectiveness of nutritional guidelines recommended by professionals depends on the veracity of the  $ME_n$  values, it is crucial to corroborate the validity of Atwater's factors and to establish their possible dependency on one or more of the parameters listed in the Introduction.

## EXPERIMENTAL DESIGN AND METHODOLOGY

The previous section focused on the quantitative aspects of Atwater's proposal and presented, as completely as possible, a critical analysis of the conceptual bases used by Atwater to obtain  $ME_n$  values. Additionally, an analysis of the procedures used to obtain the  $HC_m$ ,  $A_m$ , and  $U_p$  parameters was presented; such parameters are required for calculation of the  $ME_n$  values. Therefore, to complete the analysis, the qualitative aspects of Atwater's proposal will be considered in this section. A critical analysis of the experimental design and methodology used to obtain  $ME_n$  values is presented here, with a focus on the experimental aspects that must be improved in order to obtain a more precise and/or accurate set of  $ME_n$  values.

### Study participants

In total, Atwater and coworkers conducted 99 experiments<sup>5,11,19–21,23,24,27</sup> involving 8 participants who were studied under different controlled diets and physical activity routines. However, during more than 80% of the experimental time (257 of 318.5 days), only 3 participants were studied (for 35%, 31%, and 15% of the experimental time, respectively). By today's standards, such a number is a priori considered very low.

Moreover, in Atwater's reports,<sup>5,11,19–21,23,24,27</sup> there is no evidence that participants were selected on the basis of pre-established inclusion and exclusion criteria.

In fact, the absence of clinical and/or biochemical data for each participant (only age, height, and weight of all participants, as well as normal chest measurement and body surface area for 5 participants, were reported<sup>23</sup>) makes it impossible to justify the participant group as suitably representative of a specific population, and under no circumstance can it be considered to accurately represent a worldwide population today.

To overcome Atwater's limitations, it would be prudent to conduct new experiments in pre-established populations in which certain relevant parameters (eg, number of participants) can be statistically supported.

### Activity patterns and diets used in Atwater's studies

In Atwater's experiments, only 3 types of activity patterns were considered: (1) almost complete rest (an activity level too low to be considered usual in healthy persons; 67 experiments); (2) exhaustive exercise for 8 h/d (an exercise level too high for the common population, requiring a muscular energy consumption of between 200 and 600 kcal/d; 30 experiments); and (3) exhaustive exercise for 16 h/d (depriving the patient of an adequate amount of sleep and forcing a muscular energy consumption of approximately 1000 or 1500 kcal/d; 2 experiments). Thus, none of the experiments used activity patterns that can be regarded as representative of the current general population.

Another factor that should be reexamined is the diet used in Atwater's studies. In most experiments (63% of them), the total energy intake and/or the percent distribution of macronutrients does not coincide with current standards recommended for healthy individuals.<sup>51,65</sup> For example, for total energy intake, the *Dietary Guidelines for Americans 2015–2020*<sup>51</sup> recommend a daily intake of between 28.6 and 42.9 kcal/kg for men, whereas the European guidelines<sup>65</sup> advise an intake of 24.1 to 52.2 kcal/g. To make sure that none of Atwater's experiments are wrongly labeled as invalid, the most inclusive range (the European one) was considered. Thus, only the intakes outside the range of 24.1 to 52.2 kcal/kg were considered invalid for the purposes of this review. Before the intakes proposed by Atwater in his work experiments (in which a certain known amount of energy was used in physical activities) could be compared with the current healthy eating patterns, the ratio of intake energy to body weight had to be calculated; thus, the energy consumed through physical activity was subtracted from the actual energy intake. Considering the above, in 6% of Atwater's experiments, the intake was less than 24.1 kcal/g (including 4

experiments conducted under fasting conditions), whereas in 22% of them, the intake was too high to be considered healthy ( $>52.2$  kcal/kg). In some of Atwater's experiments, intakes exceeded 80 kcal/kg.

When analyzing the relative energy provided by each macronutrient, it is notable that, in 21% of the diets used, the energy provided by fat was less than 20% of the energy provided by all macronutrients, which is recommended by neither the American<sup>51</sup> nor the European guidelines.<sup>66</sup> Similarly, 35% of the study participants consumed a diet containing too much fat<sup>51,66</sup>; that is, the energy provided by fats was more than 35% of the energy provided by all macronutrients. In fact, in one experiment, the fat intake was as high as 64% of total energy obtained from macronutrients.

An examination of carbohydrate consumption shows that 23% of the study participants consumed an amount of carbohydrates below the recommended value, ie, less than the amount required to represent 40% of the total energy produced by the 3 macronutrients. As for total energy intake, the most inclusive range was defined so that both guidelines (the American<sup>51</sup> and the European<sup>67</sup>) could be grouped together. Similarly, 19% of the participants consumed a comparatively high amount of carbohydrates ( $>65\%$  of the energy consumed was provided by carbohydrates); as a reference, in 8 experiments, more than 75% of the energy consumed was provided by carbohydrates.

Today, current guidelines recommend that sugar intake be reduced as much as possible,<sup>51,58,67</sup> so that energy provided by sugar represents less than 10% of the total energy intake. Considering this, it is remarkable that several of Atwater's subjects (27% of them) consumed much more sugar than recommended by the "healthy eating pattern"; in 4 experiments, approximately 400 g of sucrose was supplied to the subjects each day. Furthermore, the benefits of consuming a wide variety of fresh fruits and vegetables<sup>51,67,68</sup> are widely known, yet fresh fruit was supplied in only in 3 of Atwater's experiments, and even then, in an unsuitably low amount. Moreover, fresh vegetables were not offered in Atwater's experiments. Therefore, it can be inferred that, in general terms, the fiber consumption by Atwater's participants was very low.

In 30% of Atwater's experiments, the protein intake is lower than currently recommended (worldwide guidelines<sup>51,69</sup> suggest at least 10% of total energy be provided by protein). However, in the rest of the experiments, the protein content is in line with that of currently recommended dietary patterns.<sup>51,69</sup>

The presence of alcohol in the diet represents another area of difference between Atwater's experiments and current recommendations. Today, it is generally recognized that alcohol should be omitted from the diet or consumed



in low amounts ( $<28\text{ g/d}^{51}$ ). Nevertheless, in 20 of Atwater's experiments, a comparatively high amount of alcohol was supplied to subjects daily ( $72.5\text{ g/d}$ ). At this level of intake, it is predictable that there will noticeable changes in the digestive pattern of macronutrients and, consequently, in Atwater's parameters.

On the basis of the above, 73% of Atwater's experiments were carried out under unsuitable nutritional conditions (showing total energy intake and/or the percent distribution of macronutrients to be invalid) and/or included an excessive level of alcohol consumption.

The Atwater experiments can be improved by revising the nutritional parameters or physical activity patterns used (eg, those mentioned in the Introduction) while still keeping both elements (dietary intakes and physical activity patterns) aligned with levels widely considered suitable for the general population.

### Corresponding residues of the macronutrients ingested

Previously, it was mentioned that the correct application of the equations described above requires one to know which fecal and urinary excretions contain the residues of each ingested macronutrient (ie, the corresponding residues). When classifying fecal residues (to evaluate the corresponding residues of each macronutrient ingested), the time elapsed between ingestion and excretion (digestion time), as well as the dispersive effect of the digestive process, must be considered.<sup>70</sup> As a result of the dispersive nature of digestion, the food ingested in a meal is not necessarily expelled in a single fecal excretion. Therefore, despite the use of fecal markers, a certain level of error is unavoidable when identifying the fecal excretions that contain all the residues of the first and the last meal of each experiment. Atwater<sup>5</sup> explicitly recognized this limitation. In addition, he mentioned that, usually, the first excretion included in his calculations appeared during the second or third day of the experiment.<sup>5</sup> Therefore, one would expect that, in most experiments, only 2 or 3 excretions were produced (usual experiment duration:  $\leq 4$  days). It would follow, thus, that Atwater's measurements of fecal residues included substantial inaccuracies.

With regard to urinary excretions, Atwater affirmed that "... certain part of the nitrogen in food finds its way into the bladder in a very short time (e.g., around 1 h)."<sup>5</sup> However, he also claimed that "... when the metabolism of nitrogen is increased by muscular labor, the increased excretion of nitrogen may continue for many hours after the labor has ceased."<sup>5</sup> Additionally, Atwater recognized that it is impossible to accurately estimate the delay time for urine (ie, time elapsed between intake of nutrients and excretion of

residues in urine),<sup>5</sup> and no delay time for urine was considered in any of his experiments.

In light of these factors and their effects on Atwater's results, it would be best to perform experiments over an extended time of testing (as long as possible) and to measure only average parameters (1 for each experiment). This would help overcome the problems involved in the correct designation of residues and would reduce the unavoidable inaccuracies at the beginning and the end of each experiment.

### Nitrogen imbalance

Atwater considered the above equations to be valid only when the subject is studied under conditions of nitrogen balance; that is, when the amount of nitrogen ingested is exactly the same as the amount excreted (in feces, in urine, and in perspiration that occurs during the experiment). Therefore, to estimate the validity of Atwater's data and to establish a criterion that is not overly stringent, nitrogen balance was defined to be reached when the absolute value of the difference between the amount of ingested nitrogen and the amount of excreted nitrogen is less than 0.05 times the amount of nitrogen ingested. Since some experimental imprecision is unavoidable, an imbalance of 5% could be considered acceptable. In this calculation, it is assumed (as Atwater assumed in his studies) that the correspondence between the ingested food and its residues is correct. Furthermore, to approximate the level of imbalance obtained in each experiment, two levels of imbalance could be defined: a moderate level (when the difference between the ingested and the excreted amount of nitrogen is between 0.05 and 0.15 times the amount of nitrogen ingested), and a high level (when the imbalance is  $>15\%$ ). If these criteria are applied to Atwater's experiments, 3 findings are noteworthy: (1) the nitrogen balance was reached only in 39 of the 99 experiments reported by Atwater; (2) in 43 experiments, the amount of nitrogen excreted was higher than the amount ingested, whereas only in 17 experiments, the amount excreted was lower than the amount ingested; and (3) the level of nitrogen imbalance was high in 25 experiments and moderate in 35 experiments.

It is important to recognize that, in Atwater's experiments, the correspondence between the ingested food and its residues is doubtful; therefore, the last 3 statements above can become incorrect. However, in any case, when the nitrogen inputs and outputs of studied individuals are not balanced, a certain level of inaccuracy of the estimated values for the  $F_p$  and  $U_p$  parameters could be expected.

Therefore, special care should be taken when composing the diet supplied to each study participant to

ensure that it is without protein excess or deficiency. Additionally, since nitrogen balance is impossible to achieve in certain population groups with fluctuating protein needs, it may be prudent to exclude the following population groups from such studies: infants, adolescents, pregnant women, and individuals who engage in vigorous physical activity.

### Preliminary experiments

In his preliminary experiments, Atwater focused mainly on establishing the right diet for his study participants. The diet not only had to be acceptable (palatable) to the participants but also had to be designed to achieve the desirable nitrogen balance.<sup>5</sup> Additionally, for experiments that included intense physical activity (Atwater's work experiments), the time elapsed during the digestion experiment (preliminary experiment) was used by the participant to acquire the fitness required to perform the physical routine planned for the corresponding metabolism experiment. Finally, Atwater affirmed that a finite time must elapse before a change in metabolism becomes constant.<sup>5,6</sup> He highly recommended carrying out preliminary experiments to allow the body to become adjusted to a given change (in diet and/or in physical activity type and/or level). Despite this, only 29 of his 99 experiments were preliminary ones<sup>5,11,19–21,23,24,27</sup>; that is, 41 metabolism experiments were conducted without preliminary experiments.

On the other hand, since preliminary experiments are, by definition, experiments under heterogeneous conditions, one could expect that heterogeneity would affect the results obtained. Therefore, strictly speaking, the parameters obtained in the Atwater's preliminary experiments (29 tests) should have been ignored; nevertheless, Atwater considered them as part of his results.

Thus, to ensure that the data obtained allow the estimation of a more precise and accurate set of  $ME_n$  values, it is recommended that experimental designs include preliminary experiments, the results of which are not used to calculate the  $ME_n$  values.

### Accuracy of sampling, experimental procedures, and measurements

To apply the previously mentioned equations, it is obvious that representative samples of food, urine, and feces must be collected, which requires the use of careful sampling methods and the application, when possible, of prehomogenizing techniques. Atwater, however, reported very few details about the sampling procedures used in his experiments. In at least one report,<sup>5</sup> he explicitly stated that "...compositional data were assumed rather than estimated from direct analysis of

specimens," which can greatly decrease the accuracy of the estimated parameters.

Finally, the improvements in analytical procedures and equipment over the last century make it highly probable that data obtained more recently will be more precise and perhaps more accurate than data measured 120 years ago.<sup>59,60</sup> This alone would justify new experiments focused on obtaining a more precise and/or more accurate set of  $ME_n$  values.

### CONCLUSION

Regardless of the limitations of Atwater's work, it is clear that his contributions form a founding pillar of nutritional science, representing, in his day, outstanding advancement built on the work of his predecessors.<sup>71</sup> Unfortunately, efforts to improve these contributions have been practically nonexistent for more than century, so that now, 12 decades later, it is crucial to perform a critical analysis of the experimental methodology and data that gave rise to the values currently used for  $ME_n$ . Therefore, this critical review of Atwater's works has shown number of weaknesses that highlight the likelihood of important systematic deviations in the actual  $ME_n$  values. Some of these weaknesses are as follows: (1) the doubtful representativeness of the subjects considered by Atwater, as well as the activity patterns and diets of those subjects; (2) the extremely short duration of Atwater's experiments; (3) the uncertainty related to the strict correspondence between each ingested nutrient and its fecal and urinary residues; (4) the uncertainty about whether or not the required nitrogen balance in individuals was reached during experiments; (5) the numerous experiments carried out without valid preliminary experiments; (6) the imprecision affecting Atwater's experimental measurements; and (7) the numerous assumptions, approximations, and lack of information characterizing Atwater's reports. In light of these shortcomings, it is necessary to establish new experimental procedures that permit the calculation of more precise and/or accurate  $ME_n$  parameters (specific guidelines about this have been mentioned previously in this review). It is especially important to evaluate the possible dependence of such values on certain nutritional parameters and/or physical activity patterns (see Introduction). Estimating more precise and/or accurate values for the metabolizable energy of macronutrients would allow a natural evolution of nutritional science, but most importantly, their application would allow better management of the overweight and obesity epidemic and its consequences (eg, chronic degenerative diseases), which currently affect millions of people worldwide.

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