British Journal of Nutrition (2001), **86**, 265–276 DOI: 10.1079/BJN2001404

Quantifying and separating the effects of macronutrient composition and non-macronutrients on energy density

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(Received 22 February 2000 – Revised 29 January 2001 – Accepted 10 April 2001)

The purpose of the present study was to estimate and compare the effects of macronutrient composition (relative portions of macronutrients) and of non-macronutrient components (e.g. water and fibre) on energy density (energy per unit weight) of the diets of human subjects. We used standard macronutrient energy content values to develop a simple conceptual model and equation for energy density in terms of % energy from dietary fat and % non-macronutrients by weight. To study these effects in self-selected diets of free-living subjects, we used four consecutive days of self-weighed and recorded food records for thirty-two male and thirteen female free-living adult subjects. In the range of typical human diets, the effect of % non-macronutrients by weight was several times greater than that of % energy from dietary fat, both in absolute terms and relative to daily variation in subjects' diets. Both effects were large enough to be physiologically important. Non-macronutrients (% by weight) alone explained much more of the variation in self-selected dietary energy density either between subjects (R^2 95 %) or day-to-day (R^2 95 %) than did % energy from dietary fat (R^2 5 % and 6 % respectively). Omitting beverages gave similar results. The smaller effect of macronutrient composition on energy density of diets is mainly because alterations in macronutrient composition affect only the portion of typical dietary intake that is macronutrients (one-quarter to one-third of weight). Mathematical methods are also useful in analysing observational data and for separating effects of macronutrient composition and non-macronutrients in intervention studies. These results illustrate the importance of considering non-macronutrients in the design and analysis of experimental or observational dietary data.

Diet composition: Dietary fat: Dietary intervention: Energy intake

Energy density, defined as the energy content per unit weight of food, has been found to be an important factor in the regulation of energy intake in some situations. Rolls & Bell (1998) and Blundell & Stubbs (1999) have reviewed research in this area. One fairly consistent finding that is responsible for much of the interest in energy density is that when diet composition has been covertly manipulated over a time period of hours or days, subjects have tended to eat about the same weight of food regardless of macronutrient composition or the proportion of non-macronutrient components (van Stratum *et al.* 1978; Duncan *et al.* 1983; Lissner *et al.* 1987; Tremblay *et al.* 1989, 1991; Thomas *et al.* 1992; Stubbs *et al.* 1995, 1996; Saltzman *et al.* 1997). Some contrary results have been reported. For example, Tremblay & St-Pierre (1996) noted differences in food weight across conditions when one condition involved a high-fat, alcohol-containing appetizer, Rolls *et al.* (1999) found differences in weight of intake when treatment conditions involved water in beverages *v.* water in prepared foods, and Rolls *et al.* (2000) saw differences in food weight across conditions when food volume was altered with air. Other related issues are discussed in depth by Drewnowski (1998) and Blundell & Stubbs (1999). Despite these complexities, the experimental results suggest that over periods of days or weeks, energy density is an important determinant of energy intake, and therefore that reducing energy density of foods and diets may provide an effective approach to the treatment of obesity. This approach forms the basis for the recent book *Volumetrics* by Rolls & Barnett (2000). It is therefore important to develop methods to help quantify and separate effects of dietary components on energy density.

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Energy density is affected by the macronutrient composition (relative portions of macronutrients) since fat contributes about twice the energy per unit weight as do protein or carbohydrate. Several investigators have studied the effect of dietary fat on energy density of individual foods. For instance, Poppitt (1995), Drewnowski (1998), and Blundell & Stubbs (1999) have reported that, when quantified as g fat/g food, fat is a major determinant of energy density of individual foods. Blundell & Stubbs (1999) reported, in contrast, a much weaker relation between % energy from fat and energy density of individual foods. Energy density is also affected by non-macronutrients (mainly water and fibre) because they contribute no energy per unit weight. Drewnowski (1998), Rolls & Bell (1998), Blundell & Stubbs (1999), and Rolls & Barnett (2000) have reported that the effects of these components on energy density of foods are substantial.

There has been less study of the natural variation in these quantities in daily or longer-term diets. Poppitt & Prentice (1996) showed moderate correlations between energy density and energy intake in community studies. Seagle *et al.* (1997) reported that the correlation between dietary energy density and % energy from fat in self-reported daily food intake was only modest. It is important to understand this natural variation, because it provides the context in which dietary interventions, either for experimentation or for therapy, are applied. Magnitudes of typical dietary interventions can be better understood in relation to natural dietary variation.

The studies mentioned earlier used empirical methods (statistical, mainly regression) to study effects of dietary fat and non-macronutrients on energy density. We have taken a different approach, using simple mathematical methods to quantify the effects of dietary fat and non-macronutrients on energy density due to the known differences in energy content of these components. We adopted this approach when, in the course of carrying out empirical analyses to quantify these effects, we realized that some portions of some relationships were mathematical (due to macronutrient energy content values) while other portions might represent behavioural effects of subjects, design aspects of diets, or properties of selections of individual foods. We wanted methods to help separate these types of effects. To that end, we formulated a simple conceptual model describing energy content of various components of food and used it to develop a mathematical equation quantifying the effects of macronutrient composition and non-macronutrients on energy density. In a case such as this, where some effects are driven by physical causes, the model-based approach we describe has several advantages over purely empirical methods. Effects, particularly more subtle ones, can be studied and quantified independent of small datasets and specific situations. Different ways of quantifying macronutrient composition (% weight or % energy for instance) can be clarified and compared. When macronutrients and non-macronutrients both vary, the effects of these on energy density can be separated. Dietary manipulations can be quantified in relation to each other and to the natural background variation in diets. We believe these methods will provide a useful adjunct to empirical methods in standard use.

We have developed these simple mathematical methods and used them to help answer several questions. How do macronutrients and non-macronutrients combine to determine energy density? If similar changes are made in these two factors, how do the resulting changes in energy density compare? How much of the between-subject and within-subject variation in energy density of self-selected diets selecting among typical foods is explained by these two factors, together or separately? How do the factors covary in typical diets? How do typical dietary interventions relate to this natural variation? We also use the methods to illustrate the importance of accounting for and quantifying both macronutrient composition and non-macronutrient effects in dietary interventions.

There are many other questions about energy density, including issues of physiological and sensory responses to energy density, effects on physiological and sensory responses of different methods of altering energy density, and many related questions. Questions such as these are addressed very well in the discussions by Rolls & Bell (1998), Drewnowski (1998) and Blundell & Stubbs (1999) and are outside the scope of our present paper, though the methods and results we give could be useful in studying some of these questions.

Methods

Definitions of 'food'

Recent results by Rolls *et al.* (1999) indicate that the effects of water in beverages can be quite different from the effects in prepared foods, so we carry out most analyses using both food and beverages together and using food only, omitting all beverages. Juices, soft drinks, milk shakes, etc. were classified as beverages; soups, yogurt, ice cream and other such items were classified as foods. We use the terms 'food and beverages' and 'food only' to distinguish whether beverages have been included. We use the term 'food' when methods or results are general and apply to either situation.

A conceptual model for food

Begin with some food and first divide it into macronutrients (fat, carbohydrate, protein and alcohol) and non-macronutrients (mainly water but also some fibre, and in some cases compounds such as olestra, a fat substitute). Non-macronutrients contribute 0 kJ/g. Further subdivide the macronutrients into the four types. For the purpose of estimating energy density, carbohydrate and protein have very similar energy content so we combine them (trading carbohydrate for protein or *vice versa* can have little effect on energy density). This is necessary only for simplicity, and we examine the more detailed situation later. Alcohol contributes a small proportion of energy in typical diets, and is typically not included or allowed in intervention studies, so we concentrate on the situation where the diet contains no alcohol (we later examine the effect of including alcohol). Fig. 1 shows the components of this model.

The term 'macronutrient composition' refers to the proportions of the four macronutrients in the macronutrient portion of the food, or in the simplified model to the

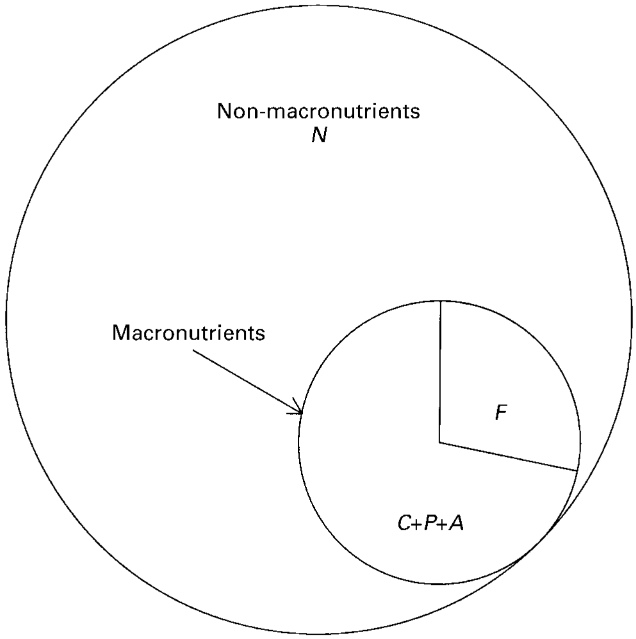

Fig. 1. A conceptual model for food showing a given quantity of food first being separated into macronutrients and non-macronutrients, and then the macronutrients being separated into fat (F) and other macronutrients $C + P + A$ = carbohydrate, protein and alcohol combined.

proportions of fat and protein+carbohydrate (i.e. the relative sizes of the slices of the smaller circle in Fig. 1). Thus, trade-offs between carbohydrate or protein and fat, or changes in the fat:carbohydrate ratio, result in changes to macronutrient composition only. The term '% non-macronutrient' refers to the percentage of a food or diet weight that is non-macronutrients. Changes in non-macronutrient components of a food or diet result only in changes to % non-macronutrient (the size of the smaller circle relative to the larger circle in Fig. 1). Trade-offs between carbohydrate, protein or fat and non-macronutrients result in changes to both macronutrient composition and % non-macronutrient.

Mathematical analysis

We developed a mathematical equation describing the earlier model. As an example of the calculations, suppose we had 1000 g food, 75 % of which by weight was non-macronutrients, and the food had 30 % energy from fat. Assuming there was no alcohol and assuming the conversions 16·7 kJ (4 kcal)/g for carbohydrate and protein and 37·7 kJ (9 kcal)/g for fat, we could vary fat (g) and protein and carbohydrate (g) combined to find that about 40 g fat and 210 g carbohydrate and protein combined gives 30 % energy from fat for the 250 g macronutrients. The total energy of this diet is 5021 kJ and the energy density is 5·021 kJ/g. The equation later describes this process mathematically. Note that no regressions or other empirical analyses are required. With the assumptions made earlier, the calculation is entirely a result of the known average macronutrient conversion factors.

For notation, let F denote the % energy from fat (100 × fat energy/total energy), referred to as % dietary fat. Standard algebra (Appendix 1) gives an equation for energy density (D) in kJ/g as a function of % dietary fat (F):

$$D(N,F) = \frac{(100-N)}{100} \times \frac{4{\cdot}184}{\frac{1}{4} + \left(\frac{1}{9} - \frac{1}{4}\right)\frac{F}{100}} = \frac{(100-N)}{100} \times D_{\mathrm{m}}(F), \qquad (1)$$

where N denotes the percentage by weight of food that is non-macronutrients (100 × non-macronutrient weight/total weight), referred to as % non-macronutrients. The expression can also be written in terms of $D_{\mathrm{m}}(F)$, the energy density of the macronutrients only, as shown earlier.

In the example earlier, $N = 75\,\%$ and $F = 30\,\%$, so substituting these values into equation 1 gives estimated energy density $D(75, 30) = (100 - 75)/100 \times 4{\cdot}184/(1/4 + (1/9 - 1/4) \times 30/100) = 5{\cdot}021$ kJ/g, as was found previously. The equation can be modified to include a term for alcohol (see Appendix 1), and the effect of this is studied later. For our main analyses we have chosen the simpler form in equation 1, using only the two key quantities N and F. Equation 1 can as easily be written in terms of % energy from carbohydrate and protein combined, using $F = 100 - (C + P)$, so that F in equation 1 is best thought of as a way of quantifying macronutrient composition.

Equation 1 separates the two main factors affecting energy density. Alterations in non-macronutrients affect only the first term $(100 - N)/100$ in the product, while alterations in macronutrient composition affect only the second term. (Note that this is not true of % fat by weight, see later.) Both N and F describe the composition rather than the amount of food. As in the conceptual model, food is first separated into macronutrients and non-macronutrients, and then the macronutrients are separated into fat and other macronutrients. The use of % energy from fat is of special interest because descriptions of diets, dietary intervention studies, and public health dietary recommendations almost universally use this measure to quantify macronutrient composition. The appearance of % non-macronutrients (N) in equation 1 is a natural consequence of this choice, and fortunately it is easily calculated and interpreted.

Equation 1 also makes it easy to compare the estimated energy density of two proposed diets. Again, for simplicity we have assumed no alcohol and equal macronutrient conversions for protein and carbohydrate. As shown in Appendix 2, if % non-macronutrients is changed from N_0 to N_1, energy density is changed by a factor of:

$$\Delta_N = (100 - N_1)/(100 - N_0). \qquad (2)$$

If % dietary fat is changed from F_0 to F_1, energy density is changed by a factor of:

$$\Delta_F = D_{\mathrm{m}}(F_1)/D_{\mathrm{m}}(F_0), \qquad (3)$$

where $D_{\mathrm{m}}(F)$ is defined as in equation 1. If both changes occur simultaneously, energy density is changed by a factor of:

$$\Delta_T = \Delta_N \times \Delta_F. \qquad (4)$$

For example, a decrease in % non-macronutrients from 85 to 75 % gives an increase in energy density by a factor of $\Delta_N = (100 - 75)/(100 - 85) = 1{\cdot}667$ or 66·7 %. Similarly, an increase in % dietary fat from 15 to 25 % gives an increase in energy density by a factor of $\Delta_F = D_\mathrm{m}(25)/D_\mathrm{m}(15) = 19{\cdot}44/18{\cdot}26 = 1{\cdot}065$ or 6·5 %. If both changes occur simultaneously, the resulting increase in energy density is the product $\Delta_T = 1{\cdot}667 \times 1{\cdot}065 = 1{\cdot}775$ or 77·5 %. Similar but more complex equations can be given if alcohol is to be included.

An equation similar to equation 1 can be derived in terms of percentages of macronutrients by weight ($f = 100 \times$ fat weight/food weight and similarly for other macronutrients):

$$D(f, c, p, a) = \frac{4{\cdot}184}{100}(9f + 4c + 4p + 7a), \qquad (5)$$

where c, p and a are the % carbohydrate, protein and alcohol respectively by weight.

Thus, energy density can be calculated from % macronutrients by weight without any further information about water or non-macronutrient content of food. This is because the non-macronutrients are contained in each of the individual percentages, through the total food weight in the denominator of each percentage by weight. These comments hold for regression as well. Furthermore, equation 5 shows that if % non-macronutrients is included in a regression along with the % macronutrients by weight, the non-macronutrients appear twice. Thus, for the purpose of separating the effects of macronutrient composition from those of non-macronutrients, equation 5 shows that the percentages by weight are not useful. For this reason as well as those previously mentioned, we will use equations 1 and 2–4 in all analyses. We will not use equation 5 in any analyses but will refer to it further in the Discussion (p. 272).

Statistical methods

In addition to equation 1, we use standard statistical methods such as means and between-subject and within-subject standard deviations to quantify typical values and variation of diets. Standard errors of estimates or quantities derived from equation 1 are not given because these have been derived mathematically rather than statistically, so statistical uncertainty information is not appropriate. R^2 values for between-subject relationships are computed as usual using multiple regression with the 4 d average diet data. R^2 values for within-subject relationships are computed using multiple regression with the daily deviations from each subject's 4 d average for each quantity of interest. Computations, statistical analyses and graphs were done in S-Plus (S-Plus Software, Version 2000 for Windows PC; Mathsoft Inc., Seattle, WA, USA).

Subjects and measurements

We use data from self-reported food intake records obtained during screening for a variety of energy expenditure studies conducted at the Center for Human Nutrition, University of Colorado Health Sciences Center, Denver, CO, USA. The forty-five subjects were thirty-two male and thirteen female healthy non-obese adult individuals not taking medications known to affect energy expenditure or food intake. Table 1 shows subject characteristics. Body composition, determined by underwater weighing, was not available for two of the forty-five subjects, but diet records were available for these two, so all forty-five subjects are included in all analyses of diet records. The Colorado Multiple Institutional Review Board approved the studies that the subjects participated in, and all subjects gave informed consent.

Table 1. Subject characteristics
(Mean values and standard deviations for forty-five subjects (thirty-two male and thirteen female))

Variable	Mean	SD	Minimum	Maximum
Age (years)	30	7·9	21	66
Height (m)	1·750	0·098	1·382	1·960
Weight (kg)	72·3	10·3	55·7	90·7
Body fat (%)	20·7	4·8	9·1	30·3
BMI (kg/m^2)	23·6	2·6	18·7	30·7

Each subject was individually trained by a dietitian in using a small food-scale to weigh and record all food and beverage intake, including beverages containing no macronutrients. Subjects were instructed to consume their usual diets during the measurement period. The food intake records were completed over four consecutive days that included one weekend day. In the presence of the subject, a dietitian reviewed the completed food intake records for clarity and completeness. Energy and macronutrient intake were determined using Nutritionist IV dietary analysis software (Version 4.0, 1994–95; First DataBank, San Bruno, CA, USA). One day of food intake data on one subject was omitted as an extreme outlier that was determined to have been due to unavailability of food (an unprepared hiking trip), giving a total of $4 \times 45 - 1 = 179$ d of diet records for analysis.

Results

Summary of diets

Descriptive statistics for the diets of the forty-five subjects are shown in Table 2 for food and beverages. Min(day) and Max(day) are the minimum and maximum daily values and Min(avg) and Max(avg) are the minimum and maximum 4 d averages. $\mathrm{SD_W}$ and $\mathrm{SD_B}$ denote the within-subject and between-subject standard deviations respectively. Table 2 also shows the corresponding data for food only. As expected, % dietary fat and energy density are higher and % non-macronutrients is lower when beverages are omitted but the overall patterns remain similar.

The conceptual model and equation 1 give accurate estimates of energy density for daily or 4 d average diets for our sample of free-living subjects

There was a maximum difference between observed energy density and energy density estimated by equation 1 of 4·6 % (0·28 kJ/g) for 4 d averages and a maximum difference of 14·9 % (0·89 kJ/g) for individual days for food and

Table 2. Summary statistics for four consecutive days of self-assessed, self-selected intake for forty-five free-living subjects*

Quantity	Mean	Min(day)	Max(day)	SD_W	Min(avg)	Max(avg)	SD_B
Food and beverages							
F (% energy from fat)	27·9	7·0	52·0	6·4	12·8	41·0	6·7
N (% macronutrients by wt)	75·0	53·2	88·2	5·6	61·4	85·4	5·3
Energy density (kJ/g)	4·92	2·17	9·47	1·12	2·77	7·45	1·05
Energy intake (kJ)	12204	3607	31711	2675	4840	25989	4362
Food weight (g)	2599	634	6910	580	1034	6020	969
Food only							
F (% energy from fat)	30·6	8·0	56·0	7·2	13·7	44·8	7·6
N (% macronutrients by wt)	62·0	37·7	79·2	7·0	51·8	72·6	4·9
Energy density (kJ/g)	7·59	4·20	12·15	1·48	5·61	9·66	1·06
Energy intake (kJ)	10327	2950	26117	2650	3554	22179	3742
Food weight (g)	1377	479	3736	320	605	3081	484

Min(day), minimum daily value; Max(day), maximum daily value; Min (avg), minimum 4 d average; Max (avg), maximum 4 d average; SD_W, within-subject standard deviation; SD_B; between-subject standard deviation.
* For details of subjects and procedures, see Table 1 and p. 268.

beverages. This accuracy is obtained even in the presence of some alcohol consumption on forty-three of the 179 study days, with individual daily consumption as high as 89 g/d. For only one of the 179 d studied (the one with 89 g alcohol consumed) did observed and estimated energy density differ by more than 9 %. Accuracy was similar for food only. The main reasons for differences between observed energy density and that calculated from equation 1 are the presence of some alcoholic beverages (in food and beverages) and differences from the average macronutrient conversions given above for specific foods or beverages in the diets. The close agreement noted above shows that these are small effects relative to the differences in energy content between fat and other macronutrients and between macronutrients and non-macronutrients.

A version of equation 1 that includes alcohol is given in Appendix 1. Using this equation gave only slightly improved accuracy with food and beverages, with a maximum difference of 4·6 % (0·28 kJ/g) for 4 d averages and a maximum difference of 8·7 % (0·50 kJ/g) for individual days. It did, however, adequately account for the single day of extreme alcohol consumption previously noted. Analyses using the methods of Bland & Altman (1986) (results not shown) show a slight tendency to over-predict energy density when energy density is high. Further slight improvements to equation 1 can be achieved by using more accurate average macronutrient conversions, but again these gains are relatively slight.

In the range of typical diets, a decrease of 10 percentage points in % non-macronutrients has a greater effect on energy density than does a 10 percentage point increase in % dietary fat

Changes in energy density for 10 percentage point changes in % dietary fat and % non-macronutrients are shown in Table 3 for various values of *F* and *N*. These values apply to either food and beverages together or food only. Throughout the range of typical diets, the changes in energy density resulting from a 10 percentage point change in % non-macronutrients are greater than those from the same change in % dietary fat. For food and beverages selected by our free-living subjects, mean % non-macronutrients was 75·0 and mean % dietary fat was 27·9 (Table 2). The next-to-last line of Table 3 shows changes in energy density related to 10 percentage point changes centred at these mean values. For food and beverages, the effect of % non-macronutrients is about $50{\cdot}0/6{\cdot}8 = 7{\cdot}4$-fold greater than that of % dietary fat. For food only, mean % non-macronutrients was 62·0 and mean % dietary fat was 30·6 (Table 2). The last line of Table 3 shows changes in energy density related to 10 percentage point changes centred at these mean values. For food only, the effect of % non-macronutrients is about $30{\cdot}3/6{\cdot}9 = 4{\cdot}4$-fold greater than that of % dietary fat. The effect of % non-macronutrients on energy density is several times greater than that of % dietary fat throughout the range of typical diets.

In the range of typical diets, a decrease of one within-subject standard deviation in % non-macronutrients has a greater effect on energy density than does an increase of one within-subject standard deviation in % dietary fat

The results in Table 3 relate to the same absolute change in the percentages *N* and *F*. However, it is not immediately clear how a 10 percentage point change in each of these quantities relates to a subject's natural daily variation. Tables similar to Table 3 can be made showing changes in energy density for a change of one within-subject standard deviation in % non-macronutrients and % dietary fat. However, examination of Table 2 shows that the within-subject standard deviations of % dietary fat (6·4 for food and beverages, 7·2 for food only) and % non-macronutrients (5·6 for food and beverages, 7·0 for food only) are quite similar. As a result, the changes in energy density per one within-subject standard deviation are again several times greater for changes in % non-macronutrients than for changes in % dietary fat.

% Non-macronutrients and % dietary fat together explain nearly all of the variation in energy density between subjects or day-to-day for our free-living subjects; % non-macronutrients explains much more of this variation than does % dietary fat

Figs. 2 and 3 show energy density (*D*) *v.* % dietary fat (*F*)

Table 3. Change in energy density (%) related to a 10 percentage point change in % non-macronutrients by weight or % energy from fat*

Mean (%)	Increase in (F) % energy from fat	Increase in energy density (%)	Mean (%)	Decrease in % (N) non-macronutrients by weight	Increase in energy density (%)
	10→20	6·2		90→80	100·0
	20→30†	6·7†		80→70†	50·0†
	30→40†	7·1†		70→60†	33·3†
	40→50	7·7		60→50	25·0
	50→60	8·3		50→40	20·0
27·9	22·9→32·9‡	6·8‡	75·0	80·0→70·0‡	50·0‡
30·6	25·6→35·6§	6·9§	62·0	67·0→57·0§	30·3§

* For details of procedures, see p. 268.
† Values are approximately those of subjects in the present study.
‡ Changes centred at means for food and beverages.
§ Changes centred at means for food only.

and *v.* % non-macronutrients (N), respectively, for food and beverages. The points are 4 d averages and the solid lines are calculated from equation 1. The most striking pattern in Figs 2 and 3 is that in these real 4 d average diets, % non-macronutrients accounts for much more of the between-subject variation in energy density than does % dietary fat. Similar graphs for food only (results not shown) show similar patterns.

Multiple R^2 values for between-subject variation are shown in Table 4 for food and beverages and for food only. The very high R^2 values show that variation from subject-to-subject in energy density is very well explained by % non-macronutrients and % dietary fat together (R^2 99 % for food and beverages, R^2 99 % for food only). Individually, the variation in energy density from subject-to-subject is explained much more by % non-macronutrients (R^2 95 % for food and beverages, R^2 85 % for food only) than by percent dietary fat (R^2 5 % for food and beverages, R^2 20 % for food only).

Multiple R^2 values for within-subject (day-to-day) variation are also shown in Table 4. The results are very similar to the situation between subjects. The daily variation in energy density is very well explained by % non-macronutrients and % dietary fat together (R^2 98 % for food and beverages, R^2 99 % for food only). Individually, the variation in energy density from day-to-day is explained much more by % non-macronutrients (R^2 95 % for food and beverages, R^2 93 % for food only) than by % dietary fat (R^2 6 % for food and beverages, R^2 11 % for food only). Graphs similar to Figs 2 and 3 can be made to show these within-subject relations. They look similar to Figs 2 and 3 so are not shown.

We repeated analyses with % energy from carbohydrate to ensure that effects of carbohydrate on energy density were not being obscured by combining carbohydrate and protein. In all cases, R^2 values with energy density were lower for % energy from carbohydrate than with % energy from fat (Table 4, last row).

The observed relationship between energy density and % dietary fat in our small sample is consistent with the higher energy content from fat

The solid line in Fig. 2 represents the theoretically calculated energy density from equation 1 as a function of

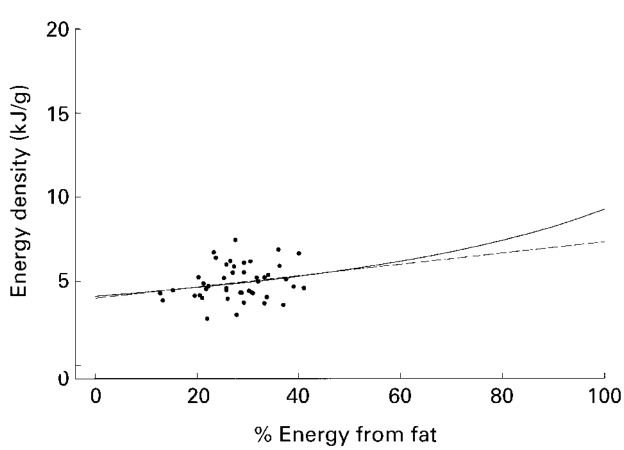

Fig. 2. Energy density (D) as a function of % energy from fat (F) for food and beverages. The points represent observed 4 d averages for the forty-five subjects described in Table 1. (—), Theoretical relationship from equation 1 (p. 267) when % non-macronutrients by weight (N) is at its mean level from the self-assessed data (N = 75·0 %); (-–-), regression line.

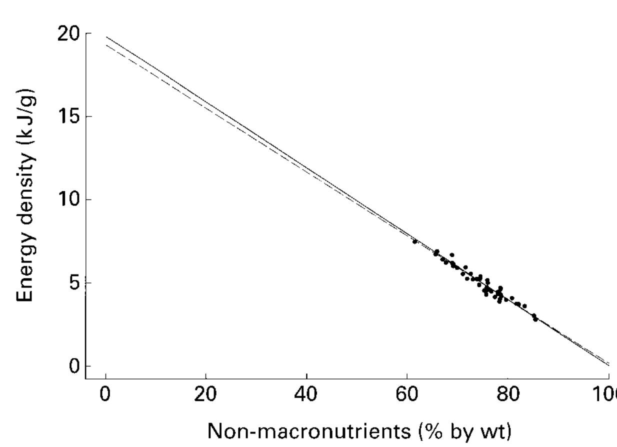

Fig. 3. Energy density (D) as a function of % non-macronutrients by weight (N) for food and beverages. The points represent observed 4 d averages for the forty-five subjects described in Table 1. (—), Theoretical relationship from equation 1 (p. 267) when % energy from fat (F) is at its mean level from the self-assessed data (F = 27·9 %); (-–-), regression line.

Table 4. Variation (R^2 values) between subjects and day-to-day (within-subjects) in energy density as explained by % non-macronutrients by weight and % energy from fat*

	Food and beverages		Food only	
Quantity	Between subjects	Within subjects	Between subjects	Within subjects
N (macronutrients by wt)	95	95	85	93
F (% energy from fat)	5	6	20	11
N and F	99	98	99	99
C (% energy from carbohydrate)†	1	1	13	2

* For details of subjects and procedures, set Table 1 and p. 268.
†Values included for comparison.

% dietary fat when % non-macronutrients takes its mean value $N = 75{\cdot}0\,\%$ for food and beverages. This line represents the mathematical effect of the higher macronutrient content of fat on energy density when N is held constant at 75·0 %. In the range of our data the empirical relationship as estimated by regression (dashed line) is very similar to the theoretical relation. The higher energy density of the higher-fat diets is consistent with what would be expected due to the higher energy content of fat. This agreement between theoretical and empirical results is not guaranteed to hold. For example, subjects who ate higher fat foods could also tend to decrease water content of their diets, which would lead to a higher energy density of the high-fat diets than could be explained by the higher macronutrient content of fat alone. This would be a behavioural effect of subjects or a result of patterns in the food supply, and it would be noted by comparison with the mathematical effect of dietary fat represented by the theoretical line. The empirical relationship in Fig. 2 is not precisely estimated in our relatively small sample, but equation 1 provides the basis for making such comparisons in larger observational studies.

We observed no correlation between % dietary fat and % non-macronutrients in our relatively small sample

Strong negative correlations between dietary fat and water content of individual foods and beverages have been reported (Blundell & Stubbs, 1999), such that high-fat foods tend to be drier. We examined our 4 d average diets for such patterns. The very small and insignificant correlation ($r - 0{\cdot}02$, P=0·89) and the random scatter in Fig. 4 show that in our data there is no indication of such a correlation in subjects' 4 d average diets for food and beverages. Results were similar using food only. Subjects who on average ate a higher % dietary fat had no tendency to eat a higher or lower % non-macronutrients.

A similar correlation on the day-to-day level can also be examined. Again, a small and insignificant correlation (r 0·04, P=0·55) and the random scatter in a graph (results not shown) show that in our data there is no indication that on days when individual subjects ate a higher % dietary fat than their average that they tended to eat a higher or lower % non-macronutrients than their average. Again, results for food only were similar. We see no evidence that the correlation sometimes stated for individual foods carries over to daily or 4 d average dietary patterns, though this could be due to our relatively small sample and resulting low power.

We note that our analyses used % energy from fat while analyses of individual foods by Poppitt (1995), Drewnowski (1998) and Blundell & Stubbs (1999) used % fat by weight (Blundell & Stubbs considered both forms). This difference is more than sufficient to explain the different results we have seen (see further discussion later).

Mathematical methods can be used to separate the effects of dietary fat and non-macronutrients in dietary intervention studies

Mathematical methods have applications in analysing and interpreting data from dietary intervention studies. Equations 2–4 allow the increases in energy density due to % dietary fat and % non-macronutrients to be quantified and separated. As an example, we consider two previously published dietary intervention studies performed for the purpose of studying the effect of dietary fat on energy intake. Stubbs *et al.* (1995) and Poppitt & Swann (1998) observed six and five (respectively) healthy male subjects under covert manipulation of % dietary fat across three levels (20, 40, 60%) using repeated-measures designs. We consider only the 20 and 60% energy-from-fat treatments. Table 5 shows that these targets were met very closely in both studies, yet the increase in energy intake was much greater in the Poppitt & Swann (1998) study (6200 kJ/d, compared with 3330 kJ/d in Stubbs *et al.* (1995)). Food weight was slightly lower on the high-fat treatments by virtually identical amounts (100 and 99 g), so this does not explain the difference in energy intake. Further examination of the published results shows that despite the similar increases in % dietary fat, energy density increased much more on the high-fat diet in the Poppitt & Swann (1998) study (by 86 %, compared with 44 % in Stubbs *et al.* (1995)). Solving equation 1 for N, we estimate that % non-macronutrients decreased from about 77·2 to 75·1 in Stubbs *et al.* (1995) and from about 78·1 to 69·4 in Poppitt & Swann (1998). These decreases of about 2·1 and 8·7 percentage points do not seem large compared with the 40 percentage point increases in % dietary fat. However, results in Table 5 indicate otherwise. Using equation 2, the increase from 20 to 60 in % dietary fat relates to an increase in energy density by a factor of about $\Delta_F = D_{\mathrm{m}}(60)/D_{\mathrm{m}}(20) = 25{\cdot}10/18{\cdot}83 = 1{\cdot}33$, or 33 % in both studies. The decrease in % non-macronutrients in Stubbs *et al.* (1995) relates to an increase in energy density by a factor of $\Delta_N = (100 - 75{\cdot}1)/(100 - 77{\cdot}2) = 1{\cdot}09$ (equation 3), while the corresponding value for Poppitt & Swann (1998) is

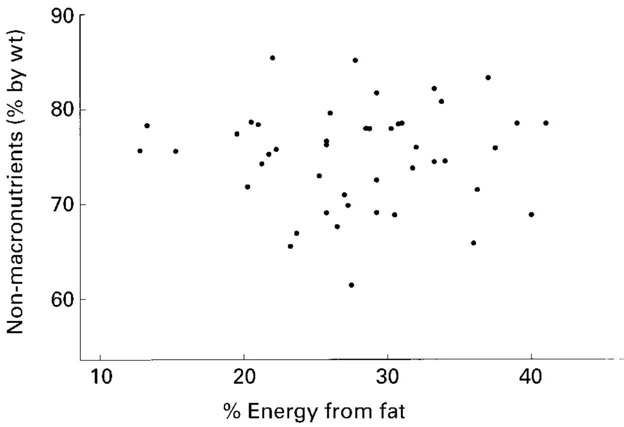

Fig. 4. Non-macronutrients (% by weight; N) *v.* % energy from fat (F) for food and beverages. The points represent observed 4 d averages for the forty-five subjects described in Table 1 (r – 0·02, P=0·98).

$\Delta_N = (100 - 69{\cdot}4)/(100 - 78{\cdot}1) = 1{\cdot}40$. Thus, in both studies, some of the observed increases in energy intake on high-fat diets were in fact due to concurrent changes in non-macronutrients. In Poppitt & Swann (1998) the relatively small increase in non-macronutrients (only about 8·7 percentage points) explained more than half of the increase in energy density and energy intake.

Examination of the published data describing the menus and foods in these studies (Table 5) shows that the observed changes in subjects' energy density were quite similar to those in the prescribed diets. Thus, the very large differences in energy intake attributed to dietary fat were in fact design properties of the diets rather than behavioural effects of the subjects. There have been reports of concurrent variation in dietary fat and non-macronutrients (see Discussion (p. 272)), so these differences between studies do not indicate that either of these studies is 'wrong'. However, they are not comparable with each other and the effects attributed to dietary fat are in fact more complex. Mathematical methods allow the separation of the two factors involved and thus clarify interpretation and comparison of the results.

Discussion

Summary of methods and results

We have given a conceptual model and mathematical equation that use the standard, known macronutrient energy content values to describe, quantify and separate the effects of dietary fat and non-macronutrients on energy density. These methods help investigators to estimate the impact on energy density of manipulations of macronutrient composition and non-macronutrients, to relate these manipulations to background variation in typical diets, and to separate mathematical and behavioural effects in experimental and observational studies. The results are of interest to investigators studying or using dietary interventions to alter food intake and/or body weight.

Using these methods, we found that in the range of diets of human subjects, changes in non-macronutrients give changes to energy density several times as great as do similar changes in macronutrient composition (% energy from fat). If a given change in energy density of a diet is

Table 5. Effects of dietary fat and non-macronutrients on energy density and energy intake in two previously published dietary intervention trials

	Low-fat treatment	High-fat treatment	Difference (high-fat−low-fat)
Subjects' intake			
F (% energy from fat)			
Stubbs *et al.* (1995)	20·6	58·7	+38·1
Poppitt & Swann (1998)	20·5	60·0	+39·5
Energy intake (kJ/d)			
Stubbs *et al.* (1995)	9030	12360	+3330
Poppitt & Swann (1998)	8600	14800	+6200
Food weight (g/d)			
Stubbs *et al.* (1995)	2100	2000	−100
Poppitt & Swann (1998)	2072	1973	−99
Energy density (kJ/g)			
Stubbs *et al.* (1995)	4·30	6·18	+44 %
Poppitt & Swann (1998)	4·14	7·68	+86 %
N (% macronutrients by wt)			
Stubbs *et al.* (1995)	77·2	75·1	−2·1
Poppitt & Swann (1998)	78·1	69·4	−8·7
Designed diets			
F(% energy from fat)			
Stubbs *et al.* (1995)	20·0	58·7	+38·7
Poppitt & Swann (1998)	20·5	60·0	+39·5
Energy density (kJ/g)			
Stubbs *et al.* (1995)	4·80	7·04	+47 %
Poppitt & Swann (1998)	5·00	8·90	+78 %
N (% macronutrients by wt)			
Stubbs *et al.* (1995)	74·5	71·6	−2·9
Poppitt & Swann (1998)	73·5	64·5	−9·0

desired, it can in general be achieved with a smaller change in non-macronutrients than in macronutrient composition (fat:carbohydrate ratio) relative to natural variation in these quantities. Even small changes in non-macronutrient content can have large effects on energy density. In daily diets of free-living subjects, non-macronutrients also explained much more of the variation in energy density than did % energy from fat. The main reason for these results is that typically most of subjects' intake (about two-thirds to three-quarters in our data) is water or other non-macronutrients and only about one-quarter to one-third is macronutrients (Table 2). Thus, the portion of food weight that is affected by alterations in macronutrient composition (trade-offs among fat, carbohydrate and protein) is fairly small. The large magnitude of the effect of non-macronutrients on energy density of diets is important in designing dietary interventions for experimentation or for weight-loss therapy.

In our sample of free-living subjects, we found that the effect of dietary fat on energy density was closely consistent with what would be expected due to macronutrient energy content differences. Our sample does not provide adequate power to precisely estimate this relationship, but our methods provide a way of estimating these mathematical effects in observational studies. We also showed that in two previously published dietary intervention studies the effect of similar dietary fat manipulations was very different from each other despite similar changes in % dietary fat, because there were different concurrent changes in non-macronutrients. These results illustrate how the mathematical methods are a useful adjunct to purely empirical methods such as comparing averages or statistical regression, because they quantify effects that are due purely to macronutrient energy content differences.

Strengths of study and methods

Our findings elaborate on the general notion that energy density is determined by fat and water because of their extreme energy content values (Drewnowski, 1998; Rolls & Bell, 1998, Blundell & Stubbs, 1999). Mathematical methods provide further information that is not available from purely empirical methods such as regression. Fig. 1 and equations 1 and 5 clarify the variables that describe macronutrient composition and % non-macronutrients. For example, Fig. 1 and equation 1 show that % energy from dietary fat and % non-macronutrients describe separate qualities of foods and diets and equation 1 shows how these combine to determine energy density. Alterations in non-macronutrients affect only the first term in equation 1. Alterations in macronutrient composition (fat:carbohydrate ratio for example) affect only the second term in equation 1. By contrast, equation 5 shows that analyses using % non-macronutrients and % fat by weight will be difficult to interpret because both components are affected by non-macronutrients. These comments relate to regression analyses as well as to the theoretical analyses we have given, and are not evident from regression alone since any combination of variables may be used in a regression model. These differences in ways of expressing dietary fat explain why in analyses of individual foods, Poppitt (1995), Drewnowski (1998), and Blundell & Stubbs (1999) found a strong effect of fat on energy density using % fat by weight, while Blundell & Stubbs (1999) noted a much weaker relationship using % fat by energy. There may well be situations such as studies of intake or body weight where total weight of fat (g) is of more interest than energy density, in which case % fat by weight may provide a better characterization of fat content of foods or diets. However, as we have noted, care is needed because this measure is affected by both the fat:carbohydrate ratio and the non-macronutrients, making interpretation difficult for some purposes.

Equation 1 also shows how dietary fat and non-macronutrients combine to determine energy density. Regression analyses using % dietary fat and % non-macronutrients assume that energy density is a linear function of these two variables (of the form $D = \mathrm{a} + \mathrm{b}F + \mathrm{c}N$). Equation 1 shows that this is not the case, that the effects are multiplicative rather than additive, and that the effect of % dietary fat is more complex (non-linear). In particular, equation 1 shows that effects of dietary fat and non-macronutrients on energy density are best stated in factor or percentage terms, a result again not evident from regression.

Equations 1–4 also show how to separate the effects of dietary fat and non-macronutrients on energy density. The model and equations also allow the separation of purely mathematical effects due to macronutrient energy content differences from those due to behaviour of subjects or design of diets. Empirical analyses can only quantify the total of these effects. We illustrated the usefulness of these methods for quantifying mathematical effects in observational data and for interpreting results of dietary intervention studies.

Limitations of study and methods

There are several limitations of our methods and results. One possible limitation of our work is the use of self-reported dietary information in our study of daily diets of free-living subjects. The mean daily % energy from fat was 28 and ranged from 7 to 52, somewhat lower than national averages. This could be due to under-reporting of high-fat foods, though we suspect it is more likely due to our subjects being volunteers for a variety of exercise and nutrition studies and thus being somewhat selected toward health-conscious individuals. Such under-reporting has no effect on the conceptual model or on equation 1: our methods quantify patterns in what subjects reported they ate. Under-reporting could affect our results concerning relative effects of dietary fat and non-macronutrients on energy density if the mean values and standard deviations of dietary quantities shown in Table 2 were highly unrepresentative of the true values. However, as Table 3 and associated results show, the effects we have described hold over a wide range of % dietary fat and % non-macronutrients, so our overall results will not have been substantially affected by mis-reporting of dietary data. For example, if the true % energy from fat eaten by our subjects was, say, 40 instead of 27·9 (the mean % dietary fat in our present data), the increase in energy density for a 10 percentage point change in % dietary fat (from 35 to 45 %) would be about 7·4 % instead of the 6·8 % reported in Table 3 (next to last line). This would not change our overall conclusions.

Another limitation is that, since carbohydrate and protein have similar energy contents, mathematical methods cannot separate effects of these macronutrients. This is similarly true of water and fibre. However, our methods could still be used to estimate the change in energy density per one within-subject standard deviation in protein and in carbohydrate (or water and fibre) separately, which would provide some further information on these effects.

A further limitation is that we would expect energy density estimates from equation 1 to be less accurate for some individual foods than for daily diets, because macronutrient energy content values of individual foods can differ substantially from the average values used in equation 1. Therefore, when calculating energy density of individual foods, item-specific energy content values should be used.

Finally, our results can only quantify effects of changes in dietary fat or non-macronutrients on energy density, but cannot provide information about how those changes might affect the sensory properties of the foods or diets or how they might affect the behaviour of subjects. Changing energy density by altering dietary fat and by altering non-macronutrients would likely have different effects on the sensory and palatability properties of foods, which would in turn influence biological responses and eating behaviours. Similarly, changes between carbohydrate and protein would have little effect on energy density but could affect other properties of foods. Drewnowski (1998) has given extensive discussion of relationships of energy density and macronutrient content with palatability and satiety.

General comments

Our finding of the greater effect of non-macronutrients compared with dietary fat on energy density of diets may seem counter-intuitive. Since the energy content of non-macronutrients (0 kJ/g) is about as much below the content of carbohydrate (16·7 kJ/g) as the energy content of fat (37·7 kJ/g) is above that of carbohydrate, it might seem that the effects of the two components on energy density should be about the same. In a diet consisting of pure carbohydrate and no non-macronutrients this would be true; replacing 1 g carbohydrate with 1 g fat or with 1 g water would change energy density by about the same amount (in opposite directions). However, a complete understanding of these effects in real diets requires both knowledge of these macronutrient energy content values plus information about typical values and daily variation of the components involved. Our data in Table 2 indicate that two-thirds to three-quarters of free-living intake is in fact non-macronutrients. Thus, alterations of macronutrient composition (changes of fat for carbohydrate or protein or *vice versa*) affect only a relatively small portion of the total food weight (the smaller circle in Fig. 1 is 25 % of the larger circle, corresponding to the average 75 % non-macronutrients for food and beverages in Table 2). This is the main reason that macronutrient composition has a smaller effect on, and explains less variation in, energy density than does % non-macronutrients in the range of typical diets.

This pattern may not hold in other situations. An extension of Table 3 shows that the relative effect of fat on energy density is larger for drier, higher-fat diets. Elementary calculus with equation 1 shows that when $F > N + 80$, the effect of dietary fat becomes greater than that of a similar change in % non-macronutrients. In particular, % dietary fat must be >80 % and % macronutrients must be <20 %, well outside the range of typical human diets. However, this would be relevant to some high-fat, low-moisture foods, and could have implications for dietary interventions in animal studies, since chow used in those studies is typically very dry.

Although we found that the effects of non-macronutrients on energy density were great, the mathematical results also show how dietary fat affects energy density. It is important to see and quantify this mathematically because it is a more subtle effect and so may not be evident in some data sets. For example, the correlation between energy density and % dietary fat in Fig. 2 is not significant (r 0·22, P=0·15), but the magnitude of the effect is close to the mathematically predicted value as shown by the similarity between the theoretical and regression lines in the region of the diets. The lack of statistical significance of the subtle fat effect may be due to the small sample size. The magnitude of the effect of % dietary fat on energy density is also in the range of physiological importance. For example, from equation 1, a decrease of 10 percentage points in % energy from fat (e.g. 40 to 30, about 1·5 within-subject standard deviations) by replacing the dietary fat (g) with the same weight of dietary carbohydrate (i.e. without any change in total food weight or non-macronutrients) translates to about a 7 % decrease in energy density. For a daily diet with energy intake of 12 200 kJ (2916 kcal)/d, about the average for our sample of free-living subjects) this translates to a decrease in energy intake of about 854 kJ (204 kcal)/d without any change in quantity (weight) of total food or of macronutrients eaten. Thus, while dietary fat does not explain the majority of variation in energy density of daily diets, even modest changes in % dietary fat can result in changes in energy density of physiological importance.

There has been some previous discussion about whether analyses of energy density are best done with diets or with individual foods. For example, Drewnowski (1998) has argued that analyses of individual foods are more meaningful because there are many combinations of individual foods that would give diets with the same energy density but these diets would have very different effects on satiety and palatability. Furthermore, accurate data on subjects' real diets are difficult to obtain and are often subject to under-reporting or other biases. On the other hand, individual foods are not completely well defined ('bread' and 'jam' *v.* 'bread and jam' for example). Furthermore, obtaining a sample of foods that truly reflects the 'universe of foods' is difficult, and selection issues can affect results. These issues do not arise with self-selected diets. The issue of defining foods *v.* beverages arises with both individual foods and with diets. Results for individual foods are useful because they relate directly to dietary manipulations and food selections that are under the direct control of researchers and consumers. Results for diets are useful because they provide the context and perspective in which such manipulations occur. We regard the study of patterns in diets and in individual foods as equally interesting, addressing different questions and useful in different ways.

There have been reports of concurrent variation in dietary fat and non-macronutrients in individual foods, such that high-fat foods tend to be low in moisture (Poppitt, 1995; Poppitt & Prentice, 1996; Drewnowski, 1998). We have also noted two instances of (different) concurrent variation in these quantities in published dietary intervention studies (Stubbs *et al.* 1995; Poppitt & Swann, 1998). There is no mathematical relationship between % energy from fat and % non-macronutrients, so such concurrent variation would be a property of the 'universe of foods' or of a particular dietary intervention. We did not see evidence of such covariation in diets of our sample of free-living subjects but we did not have adequate power to estimate this relationship precisely. This issue of concurrent variation in macronutrient composition and % non-macronutrients raises some interesting questions: if in fact there is some correlation between these two factors in the universe of foods available to consumers, does a higher-fat diet naturally lead to a lower non-macronutrient content in real diets? If such a correlation does exist in foods and/or diets, what, if any, is an appropriate concurrent variation in non-macronutrients to accompany a given change in dietary fat in order to create realistic interventions in dietary experiments?

Our theoretical and empirical results and our analyses of two previously published studies illustrate the need for food intake researchers to be aware of the relatively large effects of non-macronutrients when designing diets. In studies using smaller manipulations of dietary fat, these effects can be even more dramatic and could easily go unnoticed. For example, from Table 3, an intervention of 10 percentage points in % dietary fat would be roughly equivalent to a change in % non-macronutrients of less than 2 percentage points (the exact amounts depend on the levels of F and N), a very small difference relative to natural day-to-day variation. Thus, letting non-macronutrients 'fall where they may' in designed diets can lead to results that are difficult to interpret.

Acknowledgements

This work was supported by NIH grant DK42549 and by the Clinical Nutrition Research Unit at the University of Colorado Health Sciences Center, Denver, CO, USA (DK48520).

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Appendix 1

Derivation of equation 1

Energy is expressed in kJ, weight in g and energy density in kJ/g (4·184 kJ/kcal). Let N denote the % food weight that is non-macronutrients, ($N = 100 \times$ non-macronutrient weight/-total weight). Let F_g denote weight of fat (g), F_{kJ} denote the energy from fat (kJ), and $F_{\%}$ denote the % energy from fat, and similarly for other macronutrients. Assume macronutrient conversion factors of 16·7 kJ (4 kcal)/g for protein and carbohydrate, 37·7 kJ (9 kcal)/g for fat and 29·3 (7 kcal)/g for alcohol. Then:

$$\text{Energy density} = \frac{\text{total energy}}{\text{total weight}} = \frac{\text{macronutrient weight}}{\text{total weight}}$$

$$\times \frac{\text{total energy}}{\text{macronutrient weight}} = \frac{100 - N}{100}$$

$$\times \frac{\text{total energy}}{F_g + C_g + P_g + A_g} = \frac{100 - N}{100}$$

$$\times \frac{4{\cdot}184}{\left(\frac{1}{9}F_{kJ} + \frac{1}{4}C_{kJ} + \frac{1}{4}P_{kJ} + \frac{1}{7}A_{kJ}\right)/\text{total energy}}$$

$$= \frac{100 - N}{100} \times \frac{4{\cdot}184 \times 100}{\left(\frac{1}{9}F_{\%} + \frac{1}{4}C_{\%} + \frac{1}{4}P_{\%} + \frac{1}{7}A_{\%}\right).}$$

The second term,

$$\frac{\text{total energy}}{\text{macronutrient weight}} = \frac{4{\cdot}184 \times 100}{\left(\frac{1}{9}F_{\%} + \frac{1}{4}C_{\%} + \frac{1}{4}P_{\%} + \frac{1}{7}A_{\%}\right)}$$

represents the energy density of the macronutrient portion of the food. We denote this term D_m.

The expression for energy density can be further simplified if there is no alcohol, since then $A_{\%} = 0$ and $C_{\%} + P_{\%} = 100 - F_{\%}$ and standard algebra gives;

$$D(N, F) = \frac{100 - N}{100} \times \frac{4{\cdot}184}{\frac{1}{4} + \left(\frac{1}{9} - \frac{1}{4}\right)\frac{F}{100}} = \frac{100 - N}{100} \times D_m(F),$$

which is equation 1 (p. 267). Note that the result could as easily have been expressed in terms of $C_{\%} + P_{\%}$ using $C_{\%} + P_{\%} = 100 - F_{\%}$. This equation can be modified in the obvious way if different values of average macronutrient conversions are to be used.

Appendix 2

Derivation of equations 2, 3, and 4

As in the simplified form of equation 1 (p. 267), assume no alcohol and equal macronutrient conversions for protein and carbohydrate. If % energy from dietary fat is changed from F_0 to F_1 and % non-macronutrients by weight is changed from N_0 to N_1, the resulting energy density from equation 1 is:

$$\begin{aligned} D(N_1, F_1) &= \frac{100 - N_1}{100} \times D_m(F_1) \\ &= \frac{100 - N_1}{100 - N_0} \times \frac{(100 - N_0)}{100} \times \frac{D_m(F_1)}{D_m(F_0)} \times D_m(F_0) \\ &= \Delta_N \times \frac{(100 - N_0)}{100} \times \Delta_F \times D_m(F_0) \\ &= \Delta_N \times \Delta_F \times D(N_0, F_0) = \Delta_T \times D(N_0, F_0), \end{aligned}$$

where $D(N, F)$ is the energy density of food with % non-macronutrients by weight N and % energy from fat F, $D_m(F)$ is the energy density of the macronutrient portion of food with % energy from fat calculated as in equation 1, $\Delta_N = (100 - N_1)/(100 - N_0)$ is the factor change in energy density due to the change in % non-macronutrients by weight from N_0 to N_1, $\Delta_F = D_m(F_1)/D_m(F_0)$ is the factor change in energy density due to the change in % energy from fat from F_0 to F_1, and $\Delta_T = \Delta_N \times \Delta_F$ is the total factor change in energy density due to both changes. Similar but more complex equations can be given if alcohol is to be included.