

## **Guidelines for the Derivation of Energy Factors for Specific Food Components not already listed in Standard 1.2.8**

*Updated September 2003*

These guidelines apply to novel foods or food ingredients that are macronutrient substitutes (e.g. olestra, salatrim, d-tagatose) as well as to other individual food components that are not currently assigned a specific energy factor in Standard 1.2.8 (e.g. resistant starches, oligosaccharides).

These guidelines do not provide all the necessary information for prospective applicants wishing to apply to FSANZ to amend current standards or develop new ones.

Currently it is not possible to define or prescribe individual methods or protocols to obtain the required information to substantiate applications for energy factors for specific food components. This is because there are many different methods available and new methods are being developed. Some methods are more applicable to some types (or amounts) of food components than others, and most suffer from limitations relating to practicality, precision and/or accuracy (see Section 3).

It is recommended therefore that a variety of different methods be used to obtain the required information and that all of the different types of information are interpreted carefully together.

### **1. INFORMATION REQUIRED BY FSANZ TO SUBSTANTIATE THE APPLICATION**

Information required to substantiate an application for a specific energy factor includes:

- (a) The chemical composition and description of the food component.
- (b) If it is a mixture of components or compounds, the relative proportions of each of the compounds should be identified, together with information about any variation between different batches of the food component on the market, and the batches tested in different studies.
- (c) Measures or estimates of the gross energy (GE), urinary energy (UE), faecal energy (FE), gaseous energy (GaE) and surface energy (SE) per gram of food component should be given such that the energy factor of the component can be derived using the equation for metabolisable energy prescribed in clause 2, Standard 1.2.8 (see Section 2 for details).
- (d) Documentation of other factors that affect any of the above within a reasonable range of background diets should be presented, such as:
  - justification for and limitations of the methods used;
  - whether the GE of the food component is constant or varies with different proportions of constituent compounds;
  - whether different constituents of the food component are digested and/or absorbed differently;
  - effects of habituation/adaptation to consumption of the food component;
  - dose dependency (i.e. variations with amount consumed, how consumed such as a single large dose or several small doses, or with solids or liquids);

- the nature of background diet (e.g. high or low fat or fibre or protein);
- individual variability; and
- any other factors.

## 2. USE OF THE METABOLISABLE ENERGY EQUATION FOR THE DERIVATION OF AN INDIVIDUAL ENERGY FACTOR, AS PRESCRIBED IN STANDARD 1.2.8

The term *energy factor* describes the average amount of energy from a specific food component that is available for total (whole body) heat production and for body gains (retained energy) in humans. This is the metabolisable energy (ME) of the component. Energy factors are expressed in kilojoules per gram of food component, rounded to the nearest whole number. The factor takes into account the fact that not all of the energy ingested (gross energy or GE) is available for heat production in the body, as some is lost in faeces, urine and gases, and some may be lost from surface areas such as skin, breath and other secretions.

The equation used to calculate an energy factor is based on a definition of ME expressed as kilojoules per gram (FSANZ 2000, Amendment No. 53 to the Australian *Food Standards Code*). The equation is:

$$\text{ME} = \text{GE} - \text{UE} - \text{FE} - \text{GaE} - \text{SE}$$

where

**ME** means **metabolisable energy**

**GE** means **gross energy** (as measured by bomb calorimetry)

**UE** means energy lost in **urine**

**FE** means energy lost in **faeces**

**GaE** means energy lost in **gases** produced by fermentation in the large intestine

**SE** means energy content of waste products lost from **surface areas**.

For some food components the energy factors may be different from net metabolisable energy (NME) values listed in the literature because NME calculations deduct an additional energy component from GE: that of heat energy produced during microbial fermentation in the large intestine (heat of fermentation) and heat produced during some obligatory metabolic processes within the body, relative to glucose. For example, NME values for proteins and short chain fatty acids are lower than ME values because the energy in these compounds is used less efficiently in the body than the same amount of energy in glucose. NME values for fully fermentable carbohydrates are lower than ME values because of the heat produced during microbial fermentation in the large intestine and because the short chain fatty acids produced are used less efficiently in the body relative to glucose.

Energy factors calculated by the above metabolisable energy definition may differ slightly from ME values for the same food component listed elsewhere in the literature because a number of different equations have been used to define ME, but these differences are so small as to be of little physiological significance.

### 2.1. Measures or estimates of energy components required to derive an energy factor

The following information is required to derive an energy factor for an individual food component using the equation prescribed in Standard 1.2.8:

1. The gross energy (heat of combustion) per gram of food component(**GE**) as measured by bomb calorimetry.
2. The percentage of gross energy or the amount of gross energy per gram of food component that is completely absorbed in the upper intestine.
3. The percentage of gross energy or amount of gross energy per gram of food component that is lost in the urine(**UE**).
4. The percentage of gross energy or amount of gross energy per gram of food component that is lost in the faeces(**FE**). Faecal energy (FE) may include that portion of the food component excreted unchanged in the faeces, plus the energy content of increased microbial mass resulting from fermentation of part or all of the portion of the food component that reaches the large intestine, plus any short chain fatty acids resulting from the fermentation of the food component that escape absorption in the large intestine, plus any other metabolites of the food component that escape absorption. [*see note (a)*]
5. The percentage of gross energy or amount of gross energy per gram of food component reaching the lower intestine that is fermented [*see note (b)*], with a measure or estimate of the energy lost as gases(**GaE**). [*See note (c)*]
6. The percentage of gross energy or amount of gross energy per gram of food component that is lost from surface areas such as the skin and other secretions(**SE**). [*See note (d)*]

**Notes:**

- (a) If appropriate, FE may be divided into its separate components, including; that lost through its excretion unchanged in the faeces (uFE); that lost in microbial mass as a result of fermentation of the compound (mFE); and other losses such as short chain fatty acids or other metabolites from the food component that escape absorption (oFE).
- (b) The percentage of food component fermented could be estimated indirectly by subtracting measured amounts excreted unchanged in the faeces (uFE) from amounts *not* absorbed in the small intestine.
- (c) For fermentable carbohydrates (sugar alcohols, non-starch polysaccharides and resistant starches) the following estimates may be applied to that portion of the food component that is fermented unless different estimates can be substantiated:
- (d) 5% is lost as gases (GaE), 30% is converted to microbial mass and lost in the faeces (mFE) (Livesey, 1992). Losses from short chain fatty acids or other metabolites in the faeces (oFE) may be set at zero unless a different value can be substantiated.
- (e) Energy lost from surface areas (SE) are usually small, and may be set to zero.

Worked examples of the equation are given in Appendix 1.

### **3. EVIDENCE FROM EXPERIMENTAL STUDIES REQUIRED BY FSANZ TO SUBSTANTIATE THE APPLICATION**

Test tube (in vitro) studies and whole body (in vivo) animal studies may be used to gather preliminary data or to provide supportive or confirmative data, especially that which is not easily obtained in humans, provided that:

- data are provided to show comparability between the results of animal studies and human studies of the same or similar compounds (e.g. BNF, 1990; Livesey, 1992, Wisker et al, 1996);
- care is taken to eliminate coprophagy in rat experiments;
- experiments are done at ranges of intakes and in circumstances relevant to realistic intakes in humans; and
- clinical (human) studies are completed to confirm any preliminary data obtained by in vitro or animal experiments.

#### **3.1. Criteria**

In evaluating the evidence presented, FSANZ has adopted criteria similar to those in previous reviews (Life Sciences Research Office, 1994, 1999), and will consider studies (in vitro, animal and human) that meet the following criteria:

- have been published in peer-reviewed literature with international circulation;
- have adhered to ethical guidelines for experimentation in animals or humans (as appropriate), including informed consent in humans, and have reported details of that adherence;
- report details of funding arrangements for the study;
- report details of study design, analytical methodology, duration and statistical analysis, and that discuss the limitations of methodology used;
- report details of how the food component was administered and how ME was calculated (e.g. results from single bolus dose with ME content determined by difference, or from a range of doses and ME determined statistically using regression techniques);
- include administration of the food component orally with meals/diets of known energy and nutritional content;
- are conducted under controlled conditions where possible;
- are conducted under conditions as close as possible to the normal physiological state of the animal or human;
- in humans, use healthy subjects rather than patients with diagnosed disorders;
- use adequate (and appropriate) experimental controls;
- show appropriate statistical considerations in study design and data analysis;
- that use statistically appropriate numbers (and types) of subjects;
- use appropriate study durations;
- are minimally invasive;
- provide appropriately described details; and

- explore other factors that might affect the estimation of the energy factor of the food component such as adaptation of subjects, fasted or non-fasted conditions, ingestion as liquid or solid or with or without meals, single large dose versus multiple smaller doses, any effects of the test substance on absorption or digestion of other dietary components, and vice versa, and effects of a range of different background diets.

FSANZ will also note the number of actual studies conducted and submitted, and the consistency of the results of these studies where doses and subject characteristics were comparable.

### **3.2 Types of study**

As previously mentioned, it is not currently possible to define or prescribe individual methods or protocols to obtain the required information to substantiate applications for energy factors for specific food components. This is because there are many different methods available and new methods are being developed. Some methods are more applicable to some types (or amounts) of food components than others, and most suffer from limitations relating to practicality, precision and/or accuracy. It is recommended therefore, that applicants endeavour to access a variety of different methods to obtain the required information and that all of the different types of information are interpreted carefully together.

Methods used to evaluate energy values for non starch polysaccharides, fermentable carbohydrates and sugar alcohols have been widely reviewed (Bernier and Pascal, 1990; BNF, 1990; Ellwood, 1995; Livesey, 1990; 1992; 1993; Livesey et al, 2000; Life Sciences Research Office, 1994,1999; Van Es, 1991). Information from these references has been used to derive the outlines (below) of methods currently used to estimate metabolisable energy factors of specific food components. For substances that are not fermented, the types of study required tend to be less complicated than for substances that are fermented.

The most recent of these reviews also considered energy values for other types of food ingredients such as fat based substitutes and proteins, and is recommended reading (Livesey et al, 2000). However, be aware that all of the reviews referenced discuss the derivation of both metabolisable and net (metabolisable) energy values.

#### *3.2.1 Bomb calorimetry*

The gross energy (GE) of food components, metabolites and excreta is determined as the heat of combustion. This is measured by adiabatic bomb calorimetry, which is very precise.

#### *3.2.2 Classical dietary energy balance*

This method measures the ingested or gross energy of the compound (GE) plus that excreted in faeces (FE) and urine (UE). The method involves careful measurement and control of intake for at least several days, preceded by a period of habituation, together with collection of urine and faeces for the equivalent period. For example, if gut transit time were 72 hours, the faecal collection would commence 72 hours after the ingestion of the test substance. The method can be used in both animals and humans, although care must be taken to eliminate coprophagy in rat studies (BNF, 1990).

With this method it is possible to compare addition (or substitution) of a food component to a control diet, although it is less precise for substances that are eaten in only small amounts.

Care must be taken to ensure complete collections of faeces and urine that correspond to the intake period. To assist with this, urine and faecal markers can be used, or the experimental period can be lengthened until intake and excretion are in equilibrium. Experiments in humans are best done in a controlled environment such as in a metabolic ward.

The method is most useful for food components that are not fermented in the large intestine and which do not produce gas. However, if combined with other methods to measure the percentage of substance fermented (or gas production directly) it can also be used for food components that are fermented. In this method, energy lost in microbial mass from fermentation is already accounted for in measures of total faecal energy excretion, and the only missing measure is gaseous energy, which can be estimated from measures of the amount of the food component fermented.

Disadvantages of the method are that it is time consuming and expensive, unpopular with volunteers and practical only with limited numbers of subjects. It may give spurious results if the test substance alters the digestibility or metabolism in the gut of other dietary components, if other dietary components alter how the substance is digested and metabolised in the gut, or when intakes of the test substance are small. When combined with other methods to estimate amount of substance fermented, the results will also depend on the reliability of the adjunct methods

### *3.2.3 Isotopic tracer methods*

These methods involve the use of isotopically labelled substrates (e.g.  $^{13}\text{C}$  or  $^{14}\text{C}$ ) and measure the percent of the dose given that is recovered in metabolised form (e.g. in  $\text{CO}_2$  in breath) or in unmetabolised (urine) or undigested (faeces) form. Studies in germ free animals (i.e. no microbes to metabolise any undigested components) provide comparative data that can be used to calculate percentage fermentation in the large intestine, and hence gaseous energy (GaE). The method can also be employed with other techniques to provide adjunct information on absorption (e.g. analysis of blood glucose or other metabolites) and fermentation (breath hydrogen).

These methods are relatively quick and inexpensive, they eliminate complicated analyses of excreta, and do not require large doses of test substances. Disadvantages include:  $\text{CO}_2$  generated by colonic fermentation and host metabolism cannot be distinguished, there is a relatively high variance in results and the typical recovery of labelled  $\text{CO}_2$  is only about 60% at 48h.

### *3.2.4 Breath hydrogen test*

The breath hydrogen response is a measure of the nutrients fermented in the large intestine, and can be used to estimate gaseous energy (GaE). Basal breath  $\text{H}_2$  obtained after a dose of lactulose is compared with the breath  $\text{H}_2$  after a dose of test substance.

The test appears to give satisfactory mean values for groups of subjects, but its accuracy is questionable for a variety of reasons. These include: only one of the end products of fermentation is measured; results may be compromised by variables such as changes in diet, use of antibiotics, the type of intestinal flora, the dose given and the time period of the measurement; high doses of the test substance are required which may affect intestinal transit times; quantitative results are imprecise; and results are unreliable in individuals.

### *3.2.5 Ileal intubation and ileostomy effluent*

Ileal intubation involves the insertion of a nasogastric tube and sampling the digestive matter in the terminal ileum. Ileostomy studies involve patients who have had their large bowel surgically removed and in whom digestive excreta (from the end of the small bowel) is collected in a plastic bag. Thus, both of these methods provide a direct estimate of small intestine absorption by measuring small bowel content at the terminal end of the ileum. Combined with faecal excretion, ileal intubation also provides an indirect measure of colonic fermentation. Ileal intubation is used in both human and animal studies, although the presence of the nasogastric tube may cause abnormal intestinal absorption.

Ileostomised patients frequently have abnormal small bowel physiology including altered absorption, transit times, and the development of microbial flora at the end of the ileal segment where it exits the body. The latter is a limitation when considering fermentable food components.

Because of the invasive nature of these techniques, the results may not be quantitatively representative of normal physiologic status, but may be used to provide information on the upper limit of the amount of intestinal absorption.

### *3.2.6 In vitro susceptibility to digestive enzymes*

The ability of digestive enzymes to hydrolyse a food component may be readily determined by *in vitro* techniques. The methods are quick, inexpensive, applicable to many food components and are a useful screening technique for digestibility of food components. However, results are not quantitative, and do not necessarily reflect overall energy availability. They may also be misleading. For example, a substance might be resistant to digestive enzymes but readily fermentable by colonic microflora, or may be readily hydrolysed but not absorbable.

### *3.2.7 Other methods*

Other methods occasionally used (or in development) include:

- clinical observations of gastrointestinal discomfort as indicators of malabsorption or fermentability;
- *in vitro* methods based on anaerobic fermentation or microbial enzymic degradation;
- various comparative methods looking at absorption and blood chemistry; and
- use of germ free rats and mice in studies of fermentability.

### **3.3 Broad experimental design**

This section does not attempt to detail appropriate study designs because of the numerous different types of studies possible. Instead, it summarises some broad guidelines relating to criteria already mentioned, with some specific examples where appropriate.

#### *3.3.1 Study population*

The study population (whether animals or humans) should be clearly defined in terms of characteristics (e.g. species, age, gender, health status, normal dietary intakes, activity level if appropriate).

#### *3.3.2 Statistical analysis*

The number of subjects/animals required would be that number appropriate to achieve statistically significant differences between test and control substances. The basis for sample size calculations should be provided, especially where 'free-living' humans are used as subjects.

#### *3.3.3 Analytical methodology*

Details of methods and analytical techniques should be provided.

#### *3.3.4 Treatments*

Treatments should be described in terms of the doses, frequency, duration, mode of delivery etc. Items such as baseline or adaptation periods, treatment comparisons and control groups or substances should be described. The description of the experimental design should include study configuration (single blind, double blind, placebo controlled, cross-over etc) and randomisation procedures, if any.

Human control groups should consume diets that are similar to the average diet of the population (e.g. 10-15% of energy as protein, 30-35% of energy as fat and 45-55% of energy as carbohydrate, with 13 to 20 grams per day of dietary fibre), and/or diets that differ from the average where effects of background diet are being investigated.

Control substances should be of known ME energy content and have similar macromolecular structure to that of the test compound.

Ideally, the test food component should be incorporated into foods of the type in which it is expected to be used, and be fed in amounts and pattern that correspond to the range of expected normal consumption levels (minimum to maximum).

#### *3.3.5 Dietary energy balance studies*

Dietary energy balance studies in humans (and animals) should take into account the time required for the subjects/animals to adapt to the food component and the time required for the food component to pass through the gut. Preliminary studies should be done to ascertain the period required for adaptation to occur.

A minimum of seven days before each experimental period may be needed to adapt to some food components in humans, and a further four consecutive days (at least) should be allowed for collection of faeces. If collection periods are too short, results of faecal excretion can be highly variable depending on time and spacing of bowel movements.

To improve accuracy it is recommended that markers (e.g. radio-opaque or coloured pellets) be used to monitor the completeness of stool collection. Likewise, it is recommended that markers (e.g. para amino benzoic acid) be used to monitor completeness of urine collections.

Ideally dietary balance studies should be carried out under controlled conditions (e.g. metabolic ward). If not, the sample size should be increased to counter the expected increase in variability of results.

### 3.4. References

*Australia New Zealand Food Authority (2000) Amendment No 53 to the Australian Food Standards Code, Commonwealth of Australia Gazette (No P30 20 December 2000).*

Bernier JJ and Pascal G (1990) *The energy value of polyols (sugar alcohols)*. Medical Nutrition 26, 221-238.

British Nutrition Foundation (1990) *Energy values of complex carbohydrates. In: Complex carbohydrates in foods. The report of the British Nutrition Foundation's task Force.* Chapman and Hall, London. 55-56

Ellwood KC (1995) *Methods available to estimate the energy value of sugar alcohols.* American Journal of Clinical Nutrition, 62(suppl), 1169S-74

Life Sciences Research Office (1994) *The evaluation of the energy of certain sugar alcohols used as food ingredients.* Life Sciences Research Office, Federation of American Societies for Experimental Biology, Bethesda, MD.

Life Sciences Research Office (1999) *Evaluation of the net energy value of maltitol.* Life Sciences Research Office, American Society for Nutritional Sciences, Bethesda, MD.

Livesey G (1990) *Energy values of unavailable carbohydrates and diets: an inquiry and analysis.* American Journal of Clinical Nutrition 51, 617-637.

Livesey G (1992) *The energy values of dietary fibre and sugar alcohols for man.* Nutrition Research Review 5, 61-84.

Livesey G (1993) *Comments on the methods used to determine the energy values of carbohydrates: dietary fibre, sugar alcohols and other bulking agents.* International Journal of Food Sciences and Nutrition 44, 221-241

Livesey G, Buss D, Coussemant P, Edwards DG, Howlett J, Jonas DA, Kleiner JE, Muller D and Sentko A (2000). *Suitability of traditional energy values for novel foods and food ingredients.* Food Control 21, 249-289. Elsevier Science Ltd.

Van Es AJH (1991) *Dietary energy density on using sugar alcohols as replacements for sugars*. Proceedings of the Nutrition Society, 50, 383-390

Wisker E, Knudsen KEB, Daniel M, Feldheim W and Eggum BO (1996) *Digestibilities of energy protein fat and non-starch polysaccharides in a low-fiber diet and diets containing coarse or fine whole meal rye are comparable in rats and humans*. Journal of Nutrition, 126, 481-488.

#### 4. APPENDIX 1

Simplified examples of use of information to calculate energy factors (see Section 2 for equation and description of terms)

##### Example 1

**Theoretical food component A - not digested, absorbed or fermented, assuming that the food component has a GE of 16 kJ/g**

- None (0%) is absorbed in the upper intestine.
- None is lost in the urine(**UE=0 kJ/g**)
- All of food component is lost unchanged in the faeces, so**FE = 16 kJ/g**
- None (0%) is fermented in the large intestine(**GaE = 0kJ/g**).
- None is lost from surface areas.(**SE=0 kJ/g**)

$$\text{ME} = \text{GE (16)} - \text{UE (0)} - \text{FE (16)} - \text{GaE (0)} - \text{SE (0)} = \mathbf{0 \text{ kJ/g}}$$

##### Example 2

**Theoretical food component B - partly digested and absorbed in the upper intestine, partly excreted in the urine, partly fermented in the large intestine and partly excreted unchanged in the faeces. In this calculation, the amounts listed are assumed to have been measured unless otherwise specified.**

- Food component has aGE of 16 kJ/g
- For every 1.0 g of the food component ingested, 50% (0.5 g) is absorbed in the upper intestine, leaving 50% (0.5 g) to pass to the large intestine.
- 0.1 g (10% of the amount ingested, or 20% of the amount absorbed) is lost directly in the urine soUE= 1.6 kJ/g(10% of GE of 16 kJ/g).
- 0.395 g of the food component is lost in the faeces, soFE= 6.32 kJ/g(39.5% of 16 kJ/g. Thus 39.5% of the ingested energy, or 79% of the amount (0.5 g) not absorbed in the upper intestine that is lost in faeces(see note below).
- 0.15 g (15% of the amount ingested or 30% of the amount (0.5 g) not absorbed in upper intestine) is fermented in the large intestine. Of this amount (0.15 g), about 5% (0.0075 g, or 0.75% of that ingested) is lost as gases and 30% (0.045 g or 4.5% of that ingested) is lost as microbial matter(see note c in Section 2). ThusGaE = 0.12 kJ/g(0.75% of 16 kJ/g).
- The amount of energy lost from surface areas is zero(see note d in Section 2).(SE=0)

$$\text{ME} = \text{GE (16)} - \text{UE (1.6)} - \text{FE (6.32)} - \text{GaE (0.12)} - \text{SE (0)} = \mathbf{7.96 \text{ kJ/g} = 8.0 \text{ kJ/g}}$$

Note: In this example, the value for FE could have been measured directly (as above) or calculated from a measure of the amount of food component excreted unchanged in faeces (uFE) plus an estimate of that excreted in microbial mass (mFE)(see notes (a) and (b) in Section 2). In the above example, mFE was 0.045 g, and uFE would have been measured as 0.35 g, giving a total of 0.395 g FE. This assumes that zero energy is lost as short chain fatty acids or other metabolites(see note c in Section 2).

Methods that specifically aim to measure net energy values (rather than metabolisable energy values) have not been included in these guidelines. Such methods include: measurement of heat production (energy expenditure) by direct or indirect calorimetry, animal bioassays, growth curves, and carcass analysis studies that do not also include measurement of fractional absorption in the upper intestine and fractional excretion in the urine and faeces. These methods provide information about the efficiency with which the test substance is used in the whole body, and indirect calorimetry provides information on nutrient oxidation, but the methods do not provide information on the absorption, excretion and fermentability as required by FSANZ.