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**Variation in the apparent faecal digestibility of
macronutrients and urinary energy excretion for three diets
varying in fat and fibre content – Assessment of the Atwater
factors and related energy conversion factors**

**A thesis presented in partial fulfillment of the requirements for the
degree of Master of Science in Nutritional Science at Massey
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ABSTRACT

Background: Current systems to estimate dietary metabolisable energy (ME), often based on Atwater factors, assume that diet ME can be accurately predicted based on a few chemical components and that the ME of components is constant across foods.

Objective: Our aim was to investigate variation in apparent faecal nutrient digestibility and urinary energy excretion on different diets, and evaluate the accuracy of current systems for predicting dietary ME.

Design: The ME contents of a refined (high fat, low fibre) diet and two high fibre low fat diets were determined in balance experiments on human subjects and calculated using factorial and empirical models. Apparent faecal nutrient digestibilities and urinary energy excretions were also determined.

Results: The difference between calculated (Atwater factors) and determined ME values was up to 4% for the refined diet and 11% for the high fibre diets. The empirical models were generally no more accurate than the modified Atwater factorial model. Apparent faecal nutrient digestibility varied considerably among the three diets, as did urinary energy per unit urinary nitrogen. Mean digestibilities ranged from 81.4 (fruit and vegetable diet) to 90.0 % (refined diet) for crude protein; 87.0 (fruit and vegetable diet) to 95.7 % (refined diet) for fat; 91.1 (cereal diet) to 95.5 % (fruit and vegetable diet) for total carbohydrate. Mean urinary energy per unit urinary nitrogen ranged from 33.9 (refined diet) to 44.1 KJ/gN (fruit and vegetable diet).

Conclusion: Modified Atwater factors and some of the empirical models evaluated here may be suitably accurate ($\pm 5\%$) for use for general food labelling purposes and for determining dietary ME intakes of groups and populations, but may be inadequate for application to specialised weight-loss diets and ingredients.

KEY WORDS

Atwater factors, Digestibility, Energy, Metabolisable Energy, Urinary Energy Excretion.

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Chapter 1 Literature Review

1.1 Introduction

The present review focuses on the methods used for determining dietary metabolizable energy for humans. Topics covered include a review of the digestion of food and the absorption of nutrients in the body, and methods used for the direct and indirect determination of the metabolizable energy of food.

1.2 Defining the energy-yielding nutrients in food

Energy is required for sustaining all forms of life on earth. The prime energy source on earth is the sun. Through the process of photosynthesis, green plants intercept a portion of the sunlight reaching their leaves and capture its energy within the chemical bonds of glucose. Proteins, fats, and other carbohydrates are synthesized from this basic carbohydrate to meet the needs of the plant. Animals, unlike plants, obtain these nutrients and consequently energy, by using plants as food; carnivorous animals and humans take this process a stage further by also using other animals as food. According to Bender's Dictionary of Nutrition and Food Technology (1999), "Foods are regarded as substances which, when eaten and absorbed by the body, produce energy, promote the growth and repair of tissue or regulate these processes". The chemical components of food that perform these functions are called nutrients. Nutrients play a vital role in maintaining the basic structure of our bodies and supplying the body with the energy required to perform external activities as well as internal cellular activities. There are five major types of nutrient: carbohydrates, fats, proteins, mineral elements and vitamins. Water is also required for the maintenance of life processes. Since the function and composition of some nutrients are closely related, it is convenient to group nutrients according to their function. In this case, the major energy sources within food: carbohydrates, fats and proteins, along with alcohol and organic acids are considered as the energy-yielding nutrients for man. Their definition, constituents, nutritional functions and food sources will be discussed in detail in the following sections.

1.2.1 Carbohydrate

Carbohydrates are the main source of energy in human diets. In the diets of poor people, carbohydrate may comprise up to 85 percent of energy intake, while in the diets of the more wealthy, the proportion can be as low as 40 percent (Passmore & Eastwood, 1986). In the 19th century, carbohydrate was considered to be compounds composed of carbon, hydrogen, and oxygen following the chemical structure $C_x(H_2O)_y$, named hydrate of carbon. Since then many compounds have been identified that deviate from this general formula but retain common reactions, and thus are also classified as carbohydrates. A more comprehensive definition for carbohydrates has evolved: “carbohydrates are polyhydroxy aldehydes or ketones and their derivatives” (Pigman & Horton, 1972). Apart from covering traditional carbohydrate compounds, this definition also encompasses sugar alcohols, deoxy and amino sugars, and sugar carboxylic acids.

Chemically, carbohydrates (CHO) are commonly classified into monosaccharides, oligosaccharides and polysaccharides.

- Monosaccharides are polyhydroxy-aldehydes or -ketones, generally with an unbranched chain of carbon atoms. The most commonly occurring monosaccharides of greatest nutritional interest in food are glucose and fructose. Fructose is the sweetest of all the monosaccharides.
- Oligosaccharides are carbohydrates that are obtained from the condensation of 2 or more monosaccharides with the elimination of water to form a glycosidic link. In general, oligosaccharides contain 2-9 monosaccharide units. Disaccharides, composed of two monosaccharides, are the most common oligosaccharides, and sucrose, maltose and lactose are the best known disaccharides.
- Polysaccharides are composed of 10 or more monosaccharide units bound to each other by glycosidic linkages. These compounds are usually amorphous, colourless, almost tasteless, and vary greatly in digestibility. According to their biological function, polysaccharides can be divided into two broad groups: the storage polysaccharide starch, which is a polymer of glucose linked by α -glycosidic linkages; and the cell-wall or chemically related polysaccharides, which do not contain α -glycosidic linkages and may conveniently be called non-starch polysaccharides (NSP).

There are also other compounds, such as sugar alcohols (a group of polyhydric alcohols structurally related to sugars, where the reducing group has been reduced to a hydroxyl compound) and polydextrose (which is made by thermal polymerisation of glucose in the presence of citric acid and sorbitol), which do not fit into any of the above three categories but are still classified as food carbohydrates. They are absorbed slowly, are less cariogenic, do not encourage growth of bacteria in the mouth and are, therefore, widely used in food as sugar replacements, softeners or crystallization inhibitors. The major carbohydrates found in the human diet and their indicative digestibilities in the small intestine are summarised in **Table 1.1**.

Carbohydrates can be grouped in various ways. Based on nutritional importance, the classification of carbohydrates is focused on the digestibility of various food carbohydrates by the human body, and how much energy can be released from the carbohydrates and made available to the body. Historically, one of the first classifications of carbohydrate used in nutritional science was Atwater's total carbohydrate by difference, which is defined as the difference between 100 and the sum of the percentages of crude protein, crude fat, ash and water (Atwater & Bryant, 1899). This definition, strictly speaking, includes not only the true carbohydrates, but also some other complex forms, which are not carbohydrates from a chemical standpoint. A general calorie factor of 4 kcal per gram dietary carbohydrate has been assigned for estimating the metabolisable energy value of carbohydrate in foods since the end of the nineteenth century (Atwater & Bryant, 1900). However, growing interest in the health benefits of consuming unrefined foods led to the introduction of the dietary-fibre hypothesis by Mr Denis Burkitt and Dr Hugh Trowell in the early 1970s (Burkitt & Trowell, 1975). The term 'Dietary fibre', which was first used by Hipsley (1953), was defined as: 'The sum of the lignin and the plant polysaccharides that are not digested by the endogenous secretions of the mammalian digestive tract' (Trowell, 1972; Trowell *et al.*, 1976; Southgate, 1992). This fraction includes lignin, cellulose, hemicellulose, and soluble substances such as pectins, gums, and mucilages. The carbohydrate value used in current USA nutritional databases for calculating calories in foods is the total carbohydrate by difference minus the insoluble fibre (USDA nutrient Database, R13).

In the UK a heightened interest in the health role of carbohydrate and information needed for the treatment of diabetics, led to the introduction of two new classifications, 'available carbohydrate' and 'unavailable carbohydrate' (McCance & Lawrence, 1929). These two

Table 1.1. The principal carbohydrates in the human diet and their typical relative digestibility in the small intestine (Adapted from Englyst & Kingman, 1993; Asp, 1996).

Type of carbohydrate		Examples		Digestibility in the small intestine	
Monosaccharides		Glucose Fructose Galactose		Mostly	
Disaccharides		Sucrose Lactose Maltose Trehalose		Mostly	
Oligosaccharides		Raffinose Stachyose Verbascose Fructans		Sparsely	
Polysaccharides	Starch	Rapidly digestible starch (RDS)		Freshly cooked food	Rapid and complete
		Slowly digestible starch (SDS)		Most raw cereals	Slow but complete
		Resistant starch (RS)	Physically inaccessible starch	Partly milled grains and seeds	Resistant
	Non-starch polysaccharides	Non-Cellulosic polysaccharides	Resistant starch granules	Raw potato and banana	Resistant
			Retrograded amylose	Cooked potato after cooling	Resistant
			Cellulose	Plant cell walls	Largely resistant
			Pectins	Rhamnogalacturonans Arabinogalactans	Largely resistant
			Hemicellulose	Xylans	Largely resistant
			β -glucans	Oats	Resistant
			Gums and mucilages	Gum ragacanth Alginic acid	Resistant
Inulin		Resistant			
'New' carbohydrate food ingredients	Polydextrose		Synthetic compound	Resistant	
	Polyols	Various sugar alcohols	Sorbitol, xylitol, lactitol, maltitol	Sparsely	
	Pyrodextrins	Modified starch		Variously	

NB: Although lignin is an aromatic polymer, not a carbohydrate, it is extremely resistant to both chemical and enzyme degradation and usually analysed as part of dietary fibre.

concepts were further developed by Southgate (1973). Available carbohydrates are the starch and most of the sugars (monosaccharides and disaccharides). They are broken down by intestinal digestive enzymes into simple sugars (monosaccharides), then absorbed in the small intestine. They are the main source of energy available to tissues. Unavailable carbohydrates represent non-starch polysaccharides (such as cellulose, hemicellulose, gums and pectin) plus lignin that cannot be digested by the human alimentary enzymes. This material passes unchanged into the colon where it is fermented to a variable extent by the commensal microbiota. Gases produced by microbial fermentation may be passed as flatus or absorbed and excreted through the lungs. The short chain fatty acids produced during the fermentation process are absorbed, providing a source of energy. Both 'available carbohydrate' and 'unavailable carbohydrate' may be determined directly (Southgate, 1969a & b, 1981), and values have been adopted into food composition tables and nutritional databases. The value of 3.75 kcal per gram available carbohydrate (expressed as monosaccharides) is used for estimating the metabolisable energy value of foods (Southgate 1974; Paul & Southgate, 1978; Holland *et al.*, 1991).

In the New Zealand food tables, only available carbohydrate is taken into account when calculating the energy contribution of food carbohydrates. Carbohydrates are classified as available carbohydrate (which is defined as the sum of the individual mono- and disaccharides and starch, expressed as the weight of the carbohydrate) and dietary fibre (the total non-starch polysaccharide in the foods). The calorie conversion factor adopted is 4 kcal per gram available carbohydrate (Athar *et al.*, 2003).

Carbohydrates provide energy, fibre and naturally occurring sweeteners to the body; however, the main nutritional function is to supply energy. Available carbohydrates supply energy to the body relatively efficiently. When enough carbohydrate is provided to meet the energy needs of the body, protein can be spared or saved to use for specific protein functions. When carbohydrate intake is insufficient, fat is metabolised to provide energy for the body's needs. However, excess ketones, the intermediate products of fat metabolism, can overwhelm the body's physiological system and ketoacidosis can develop, a condition which can be lethal if uncontrolled. Although lipids and proteins can, if necessary, provide energy for most bodily needs, the brain and nerve tissues function best on glucose from carbohydrates. However, over-consumption of available carbohydrate, especially excessively sugared food, may lead to major health concerns such as: nutrient displacement,

dental caries, obesity, diabetes and hyperactivity. In contrast to available carbohydrate, dietary fibre is not primarily a source of energy, but is important for maintaining gut function. High-fibre diets may reduce the efficiency of enzyme hydrolysis in the upper gastrointestinal tract and slow the rate at which glucose enters the bloodstream, and are used for formulating energy-controlled diets and diets for the control of diabetes. Dietary fibre also increases the bulk of the faeces and the rate of passage of material through the large intestine (Passmore & Eastwood, 1986). The risk of developing obesity, constipation, haemorrhoids, diverticular disease, and colon cancer may be decreased by regularly consuming sufficient amounts of dietary fibre (Trowell, 1972; Spiller & Kay, 1980; Royal College, 1980; Southgate, 1992). The most recent dietary guidelines recommend increasing the consumption of complex carbohydrates. The Food Pyramid (USDA, 2006) suggests 6 to 11 daily servings of grains and 5 to 9 daily servings of fruits and vegetables in order to reduce fat intake and increase the intake of starch and dietary fibre. There is considerable debate, however, as to the calorie conversion factors that should be used for estimating the energy value of carbohydrate, and the contribution dietary fibre makes to human dietary ME intake.

Concentrated food energy sources of carbohydrates are grains, legumes, and starchy root vegetables. Dietary fibre is available in many foods, especially in fruits, vegetables, and whole grain products.

1.2.2 Fat

Fat is the densest form of stored energy in both food and body tissues. It provides over twice the amount of energy in kcalories per gram as does carbohydrate or protein. Surprisingly, there is no precise definition for the word 'fat'. The term is generally applied to those foods that are obviously fatty in nature, greasy in texture and immiscible with water. Biochemists, in an effort to be more precise, have coined the term 'lipid' to describe a large group of naturally occurring fat-like substances, whose members are often unrelated physiologically or chemically, but are all insoluble in water, and soluble in solvents such as chloroform, hydrocarbons, alcohols or ethers (Gunstone & Norris, 1983; Gurr & Harwood, 1991; Gurr, 1992). 'Total lipids' refers to the sum of mono-, di-, and triglycerides, free fatty

acids, phospholipids, glycolipids, terpenes, sterols, waxes, and other ether-soluble compounds (Linscheer & Vergroesen, 1988). In nutrition and dietetics, fats are a subclass of lipids, but 'fat' is often used interchangeably with the term 'lipid' (Aurand *et al.*, 1987). The U.S. Food and drug Administration (FDA) food labelling regulations, published in January 1993, defined fat for nutrition labelling purposes as the sum of fatty acids expressed as triglyceride equivalents, which takes into account all the possible sources of fatty acids in a food. The fat content of food reported in food composition tables refers to the weight of the substances extracted from ether, which includes true fats (triglycerides), free fatty acids, sterols, chlorophyll and some other pigments. Here the word 'fat' is used when referring to the fat content of food and diets, but follows the chemical nomenclature when considering the metabolism of lipids in the body.

Unlike carbohydrates, lipids are not polymers but rather are molecules extracted from animal and plant tissues. They are a heterogeneous group of compounds and can be classified into three groups: fats or triglycerides, the fat-related substances of phospholipids and sterols (Grodner *et al.*, 1996). About 95% of the lipids in foods and in our bodies are in the form of fat as triglycerides (Hilditch & Williams, 1964). Therefore, triglycerides have been the major nutritional concern in the diet. A typical triglyceride molecule consists of three long or medium chain fatty acids esterified with glycerol. **Figure 1.1** shows a typical example: a saturated fatty acid (C16:0) is at position 1 on the glycerol molecule, an unsaturated fatty acid (C18:1) in position 2. The fatty acid in position 3 can be either saturated or unsaturated. The type of fatty acids present in a triglyceride determines not only the physical characteristics of the fat, but also the storage stability and nutritional value of the foods which contained that fat (Passmore & Eastwood, 1986).

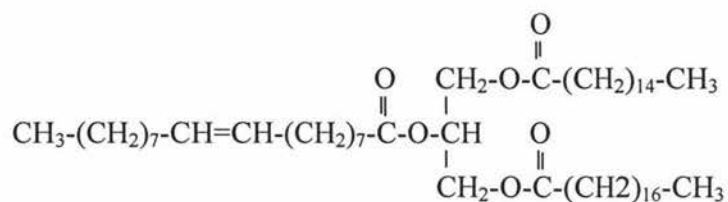


Figure 1.1. Structural formula of a typical triglyceride

Over 40 different fatty acids (FA) are found in nature, they have a basic formula $\text{CH}_3(\text{CH}_2)_n\text{COOH}$ where n can be any even number from 2 to 24. According to the number of double bonds between the carbon atoms, fatty acids can be divided into three groups:

saturated FA (SFA), monounsaturated FA (MUFA) and polyunsaturated FA (PUFA). Saturated fatty acids contain no double bonds, while monounsaturated fatty acids contain one and polyunsaturated fatty acids contain two or more double bonds. Among all fatty acids, polyunsaturated fatty acids are of particular interest in human nutrition. **Table 1.2** lists the nomenclature and sources of the most important fatty acids found in foods.

Table 1.2. Nomenclature for the more important natural fatty acids. (Adapted from Passmore & Eastwood, 1986 and Thomas, 1994).

Carbon: double bonds	Common name	Systematic name	Common natural sources
Saturated acids			
4:0	Butyric acid	Tetranic acid	Coconut oil and dairy products
6:0	Caproic acid	Hexanoic acid	Coconut oil and dairy products
8:0	Caprylic acid	Octanoic acid	Coconut oil and dairy products
10:0	Capric acid	Decanoic acid	Coconut oil and dairy products
12:0	Lauric acid	Dodecanoic acid	Coconut oil and dairy products
14:0	Myristic acid	Tetradecanoic acid	Coconut oil and dairy products
16:0	Palmitic acid	Hexadecanoic acid	Palm oil, cottonseed oil, butter, meat fat
18:0	Stearic acid	Octadecanoic acid	Meat fat, butter, chocolate
20:0	Arachidic acid	Eicosanoic acid	Nut and seed oil
22:0	Behenic acid	Docosanoic acid	Peanut oil, peanuts
Monounsaturated acids			
16:1 n7	Palmitoleic acid	9 cis-hexadecenoic acid	Cod liver oil, meat fat, fish
18:1 n9	Oleic acid	9 cis-octadecenoic acid	Olive oil, nut & seed oils, meat fat, butter, eggs, avocado
22:1 n9	Erucic acid	13 cis-docosaenoic acid	Rapeseed oil
Polyunsaturated acids			
18:2 n6	Linoleic acids	9,12 cis, cis-octadecadienoic acid	Vegetable oils, nuts, lean meat, eggs
18:3 n3	Alpha-linolenic acid	9,12,15 all cis-octadecatrienoic acid	Soyabean & rapeseed oils
20:4 n6	Arachidonic acid (AA)	5,8,11,14 all cis-eicosatetraenoic acid	Offal, game, lean meat, egg
20:5 n3	Timnodonic acid (EPA)	5,8,11,14,17 all cis-eicosapentaenoic acid	Fish oil
22:5 n3	Clupanodonic acid (DPA)	7,10,13,16,19 all cis-docosapentaenoic acid	Brain, liver
22:6 n3	DHA	4,7,10,13,16,19 all cis-docosahexaenoic acid	Fish oil

The physical properties of fatty acids are related to their chemical composition. Water solubility depends on chain length, while the melting point is related to both chain length and degree of unsaturation. Vegetable oil has more long chain polyunsaturated and fewer short chain fatty acids than hard animal fats. Fish oil, on the other hand, tends to have more polyunsaturated fatty acids, especially those long chain polyunsaturated fatty acids of the omega 3 (ω -3) type, such as eicosapentaenoic acid (EPA C20:5) and docosahexaenoic acid (DHA C22:6). The biological function of fatty acids is also related to their chemical

composition. There are two fatty acids, *linoleic acid* and *alpha-linolenic acid*, known as dietary essential fatty acids that play an important role in the human body. They cannot be made by the human body and so must be supplied by food. Overall, the functions of fat from diet can be divided into the following four major categories:

Source of energy: Fat serves as the most compact energy source available for body metabolism. It has a higher energy value than either carbohydrate or protein. A general calorie factor of 9 kcal per gram fat is used for estimating the metabolizable energy value of fat in foods.

Palatability: Fat makes food smell and taste good. Bread with butter (margarine), salad with dressing, desserts with cream are all examples where fat makes foods taste and feel pleasant for many people.

Satiety: Fat is calorifically dense, it slows down digestion and can make people feel full and satiated.

Nutrient source: Fats provide the body with essential fatty acids (EFAs). Essential fatty acids have important functions in the body. They form part of the structure of all cell membranes, help in the regulation of cholesterol metabolism, and provide the raw materials from which the hormones known as prostaglandins are made. Fat is also essential for supplying fat-soluble vitamins, such as vitamin A, D, E and K, to the body. These vitamins play an important role for some physiological functions in the body.

Fats are found in almost every natural food. Generally, the fat content is low in fruits and vegetables, but high in meat, milk, cheese, eggs, and table spreads. Nuts, olives and avocados are rich sources of fat, containing as much as 70% of fat. Most vegetable oils and some fish oils are good sources of essential fatty acids. Minimal requirements for fat may indeed be very low, but EFA intake must provide 1 to 2 percent of total dietary energy. Expert committees currently recommend that linoleic acid should provide at least 1 per cent of the total daily energy intake and that alpha-linolenic acid should provide at least 0.2 percent of the total daily energy intake (DoH 1991). Although there is no evidence that a high dietary intake of PUFAs is associated with any direct threat to health, it is nevertheless recommended that dietary intake of PUFAs should not exceed 10 percent of total food energy (Fox & Cameron, 1995).

High fat diets are almost always high in energy, and are usually associated with high plasma cholesterol levels, especially low density lipoproteins (LDL), which may lead to a variety of

diseases. Like saturated fats, the intake of *trans* fat increases the risk of coronary heart disease. Therefore, the Diet and Health dietary guidelines (NRC, 1990) advise that the caloric intake from fats should be 30% or less with 10% or less of calories coming from saturated fats, and caloric intake from trans fats should be less than 1% (WHO, 2002).

1.2.3 Protein

Protein generally comprises about 10-15% of dietary energy, but its significance is more than simply that of an energy source. It is the major functional and structural component of all the cells of the body. The word protein originally comes from the Greek word *proteios*, which means of the first rank or importance. However, the understanding of protein nutrition has taken centuries to achieve its current standing (Carpenter, 1994). Nowadays, proteins are considered as macromolecules formed by linking many smaller molecules namely amino acids in various combinations. Amino acids (like glucose) are organic compounds made of carbon, hydrogen, and oxygen. However, amino acids also contain nitrogen, which clearly distinguishes protein from other nutrients. The general formula for an amino acid is shown in **Figure 1.2**.

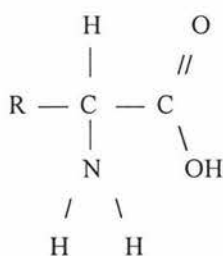


Figure 1.2. *The general formula of an amino acid. R represents the side chain, which determines the specific characteristics of each individual amino acid.*

There are approximately 20 amino acids from which all proteins are made that are required by both plants and animals. The human body can synthesize some of the amino acids for its own protein-building function, but nine of the amino acids cannot be made by the cells of the body and must be obtained from food: digested, absorbed, and then brought to cells by circulating blood. They are known as dietary essential amino acids, including histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. In order to build proteins, a constant supply of 20 amino acids, especially the 9 essential

amino acids, must be maintained in the body. If the body is in short supply of essential amino acids, it will break down tissue proteins to create what it needs. Therefore, if the diet supplies an essential amino acid in a quantity below that needed, protein synthesis will be impaired. From the view of nutritional importance, the quality of different proteins in foods depends on their amino acid composition. A complete protein is one that contains all nine essential amino acids in amounts and proportions best suited to maintain life, and support growth when used as the sole source of protein. Animal-related foods, such as meats, eggs, milk, and most of dairy products, are all good sources of complete protein. Soya beans are the only plant source that provides all nine essential amino acids in a balanced way. Proteins from other plant foods, such as vegetables, grains, legumes, nuts and seeds, are incomplete proteins which are limited in one or more essential amino acids. Eaten together, however, plant foods can offset each other's limiting amino acids and provide the full array of amino acids needed without the fat associated with meat or dairy products. The proportion of the energy provided by protein in the foods is often used as an index to assess whether foods are poor, adequate or good protein sources.

The protein content of foods is estimated based on the quantity of nitrogen present. In many foods, nitrogen comprises 16% of the total weight of protein, therefore, the protein content can be calculated by multiplying the nitrogen value obtained from chemical analysis by 6.25 for meats and most other foods. Because the nitrogen content of some food proteins may be greater or less than 16%, the factors used for converting grams of nitrogen to protein of these foods vary. **Table 1.3** lists some special factors used for converting nitrogen to protein in foods.

Table 1.3. Special factors for calculating protein from the total nitrogen content of food¹.

Food		Factors	Food		Factors
Animal origin	Milk	6.38	Legumes	Soya beans	5.71
Grains & cereals	Whole-wheat flour	5.83		Peanuts	5.46
	White flour	5.70	Nuts	Brazil	5.46
	Rye	5.83		Almond	5.18
	Rice	5.95		others	5.30
	Oat	5.83	Seeds	Sesame	5.30
	Corn (maize)	6.25		Pumpkin	5.30
	Barley	5.83		Flaxseed	5.30

¹ Adapted from table 3 of Agriculture Handbook No. 74, revised edition, February 1973 (Merrill & Watt, 1973).

The primary function of food proteins is the provision of amino acids for the production and maintenance of body proteins (including enzymes), however, excess amino acids from protein are typically transaminated and the non-nitrogenous portion of the molecule transformed into glucose (gluconeogenic amino acids) and used directly for energy or stored as fat or to a lesser extent as glycogen. The unneeded nitrogen is converted to urea and excreted in the urine. The secondary role of proteins is, therefore, as a supplementary energy source. Unlike carbohydrate and fat, protein is incompletely oxidized in the body. Nitrogenous substances, such as urea, uric acid and creatinine, arise from the process of the metabolism of all amino acids, and are excreted in the urine. Observations of the heat of combustion of urine have shown that it contains unoxidised material equivalent to 33.1 kJ (7.9 kcal)/g of nitrogen or 5.23 kJ (1.25 kcal)/g of protein oxidised by the body (Merrill & Watt, 1973). Further undigested dietary proteins along with desquamated intestinal mucosa cells and bacterial nitrogen and mucin are also lost in faecal excretion. Atwater concluded from his experiments that the net absorption of nitrogen from food protein was 92%, and a general calorie factor (Atwater factor) of 4 kcal per gram was assigned to the total amount of protein in the diet for estimating the metabolisable energy value of foods (Merrill & Watt, 1973).

All of the protein and amino acids (except for the free amino acid pool) in the body serve either a structural or metabolic function. Unlike carbohydrates and fats that can be stored in the form of triglycerides and glycogen, there is no storage form of protein or amino acids. Body proteins are in a constant state of flux and are continually being degraded and resynthesized. Therefore, proteins in the diet are the only source to supply the amino acids required for the growth of babies and children and also the amino acids needed for the maintenance of tissues in adults. If the energy content of the diet is inadequate, protein is also used to supply energy. Sherman in 1920 reviewing all the evidence from European and American sources, concluded that the minimum amount of protein required to maintain N equilibrium lies between 21 and 65g / 70 kg body weight / day (Sherman, 1920). Currently the amount of protein required daily has been recommended as approximately 50 g per day for healthy young adult females and approximately 60 g per day for healthy young adult men, provided it is of good quality (NRC, 1989).

1.2.4 Alcohol

Ethyl alcohol (ethanol: C_2H_5OH) is the principal alcohol of nutritional significance in nature, and has been produced by the fermentation of sugar and used by man for centuries. It may serve as a disinfectant, a drug, a beverage or a preservative. In classical experiments using a human calorimeter, Atwater and Benedict showed that the energy liberated by oxidation of ethanol can be utilised by the body and that its use replaces similar amounts of energy derived from carbohydrate and fat (Atwater & Benedict, 1902). The gross energy value of alcohol is normally 7.07 kcal (29.7 kJ) per gram. Results from respiration calorimeter studies showed that 98 percent of the ingested alcohol energy could be used by the human body for both muscular work and maintaining body temperature. Atwater and Benedict assigned a general calorie factor of 6.9 kcal (29kJ) per gram for alcohol to be used in food tables to estimate the food energy value. However, later work on energy metabolism of alcohol by Prentice and his group has suggested that the utilization of ethanol may not be very efficient and the useful energy content of ethanol might be considerably lower than its suggested ME value (Prentice *et al.*, 1992; Sonko *et al.*, 1994; Rumpler *et al.*, 1996).

Unlike most foods, alcohol can be absorbed by the body without prior digestion. Absorption of alcohol takes place mainly in the small intestine but also through the walls of the stomach. It provides a ready and more concentrated source of energy for the body than either carbohydrate or protein. However, the oxidation of alcohol in the body is very slow and on average only about 7 g can be oxidised in an hour, which limits its contribution to energy needs. Alcohol is also a drug and can affect the central nervous system. How alcohol intake interacts with the physiological control of food intake remains unclear, although, an excessive intake of alcohol can have a damaging effect on health. If alcohol replaces fat as the main energy source for the liver, metabolism of fat declines and lipid accumulates in the liver producing what is known as a 'fatty liver'. Body weight gain in young men in the 20-30-year-old group has been linked with the onset of frequent drinking. There is also some suggestion (Dallongeville *et al.*, 1998) that alcohol consumption (and smoking) is particularly conducive to fat deposition in the abdominal area, thus producing a selective increase in the waist-hip ratio with all of its associated hazards. In addition alcohol may impair the absorption of B vitamins and other nutrients (Fox & Cameron, 1995).

1.2.5 Other food components

Protein, carbohydrates, fat and alcohol are the major components of foods that contribute calories to our diet. However, there are also other components of foods, such as organic acids, polyols, isomalt, and polydextrose, which also contribute calories, but usually on a much smaller scale.

Organic acids are widely distributed in foods, but only contribute a significant amount of energy for a few foods, such as fruits (e.g. lemon), fermented foods (vinegar) and so on. Estimated caloric amounts given for acetic, citric, malic, and lactic acids are 3.49kcal (14.6kJ), 2.47kcal (10.3kJ), 2.39kcal (10.0 kJ) and 3.62kcal (15.1kJ) per gram, respectively (Athar *et al.*, 2003). Oxalic and tartaric acids are not included in food tables because they are not metabolised in the body in significant quantities.

Polyols, isomalt and polydextrose are a new group of food ingredients classified under the carbohydrate category. They are generally absorbed from the gut more slowly and used less efficiently by the human body. Very small amounts of polyols, isomalts and polydextrose occur naturally in foods, but they are now widely used as sugar and fat substitutes to produce low calorie foods. There is no agreed approach at an international level for assigning a consistent factor for polyols. However, the EC 1993 Nutrition Labelling Directive prescribes a generic factor of 10 kJ/g for all polyols (CAC, 1993; CEC, 1993), whereas New Zealand regulations assign individual energy factors for each component: 1 kJ/g for erythritol, 18 kJ/g for glycerol, 11 kJ/g for lactitol, 16 kJ/g for maltitol, 9 kJ/g for mannitol, 14 kJ/g for sorbitol and xylitol, respectively (ANZFA, 1999). The same principle that applies to polyols has been applied to isomalt and polydextrose. The energy factor assigned to isomalt, therefore, is 12 kJ/g, and 5 kJ/g for polydextrose. In general, these compounds contribute only a small amount of energy (2-4%) to the total diet and to an individual's overall energy intake (Livesey & Elia, 1995).

1.3 Metabolism of energy in the body

Energy provides the capacity to grow, survive and perform work. However, energy is a physical concept. People do not eat 'energy' but rather nutrients, therefore the metabolism

of energy in the body is reflected by the utilisation of energy-yielding nutrients in the body. A brief discussion on how the energy supplied in the diet is utilised by the body will be given in the following section.

1.3.1 Definition of energy terms

Over the years the meanings of some terms used in a discussion of the energy value of foods have changed, so in the following the terms of most importance are explained. An overview of the possible fates of food energy utilisation and the nutritional terms used in energy metabolism in the body are given in **Figure 1.3**.

1. **Ingested energy** = Gross energy (GE)
 - ↓ → Faecal energy (FE) (include endogenous energy additions to the gut)
2. **Apparent Digestible energy** (DE)
 - ↓ → Urinary energy (UE)
 - ↓ → Surface energy (SE)
3. **Atwater's Metabolisable energy** (AME)
 - ↓ → Gaseous energy (from microbial fermentation) (GaE)
4. **True Metabolisable energy** (TME)
 - ↓ → Heat of microbial fermentation
 - ↓ → Obligatory thermogenesis
5. **Net Metabolisable energy** (NME)
 - ↓ → Non-obligatory dietary thermogenesis
 - ↓ → Thermogenesis due to effects of cold, drugs, hormones
6. **Net energy** (NE)
 - ↓ → Energy for basal metabolism (maintenance)
 - ↓ → Energy for physical activity
 - ↓ → Energy for body gains

Figure 1.3. Overview of food energy utilisation in the body (adapted from FASEB, 1994).

Gross energy (GE) represents the total energy ingested from a food and is the heat of combustion of the food. It is readily determined by burning samples of food in a bomb calorimeter and is presented in caloric units. The calorie is a derived, not a basic unit. A

calorie is the amount of heat required to increase the temperature of 1 g of water from 14.5 to 15.5°C at a pressure of 1 atmosphere. The SI unit of energy is the joule (J), which is defined as the energy used when 1 kilogram (kg) is moved 1 metre (m) by a force of 1 newton (N). The calorie and joule is convertible. The conversion factor is 4.184, i.e. 1 kcal = 4.184 kJ, or 1 kJ = 0.239 kcal (Royal Society, 1972).

Digestible energy (DE) is the total ingested energy minus the energy content of the faeces. Faecal energy not only comes from undigested nutrients, but also includes energy arising from desquamated intestinal mucosa cells and various organic substances secreted or excreted into the intestinal tract. Therefore, the digestible energy is an approximate estimate of the energy absorbed by the body and is more often referred to as *apparent digestible energy*. *Apparent digestibility* (coefficient of digestibility) can be calculated (Merrill & Watt, 1973) as follows using nitrogen for purpose of illustration:

$$\text{Apparent N digestibility} = \frac{N \text{ in food} - N \text{ in faeces}}{N \text{ in food}} \times 100$$

Apparent digestibility is usually a slight underestimate of true digestibility as faecal excretion includes not only the undigested dietary nutrients but also the endogenous secretions, such as exfoliated intestinal cells, residues from digestive enzymes and lubricants, and bacterial mass.

Metabolisable energy (ME) is the quantity of energy entering the body in useful form. It is used to fuel productive processes, to maintain or add to the tissue mass, to enable various forms of work to be performed, and to provide necessary heat to the body. Metabolisable energy is less than digestible energy because some additional losses beyond those in the faeces must be accounted for. Energy-containing substances, such as urea, uric acid and creatinine, are lost in the urine due to the incomplete oxidation of protein. Small amounts of energy are lost from hair, skin and other secretions (surface energy). There are also losses of energy via breath and flatus, mainly in the form of hydrogen and methane gases. However, surface losses and gaseous losses have traditionally been thought of as negligible. Therefore, the traditional definition of *Metabolisable energy* by Atwater (point 3, Figure 1.3) is the amount of dietary energy which is absorbed into the body and not excreted as energy containing organic compounds via the faeces or urine, i.e. ME = GE – FE – UE

(Merrill & Watt, 1973). It is now known that fermentation in the large intestine can be quantitatively significant for some foods. Therefore, a current definition of *metabolisable energy* is the ingested energy minus energy losses in faeces, urine and surface and combustible gases, and is referred to as *true metabolisable energy* in the Australia New Zealand Food Standard Code (ANZFA, 1999). This definition of ME is similar to that used by Schutz (1984) and Sentko (1992) and describes approximately the amount of energy available for total (whole body) heat production and for body gains (tissue or milk synthesis, energy stores) (Allison & Senti, 1983). It includes the energy available for heat of fermentation. Heat of fermentation is not necessarily an energy loss to the whole body as humans are warm-blooded animals and heat produced from fermentation may contribute to thermoregulation.

Net metabolisable energy (NME) is the metabolisable energy ingested that is not wasted as heat. The heat of fermentation and heat of metabolism spent in yielding 'high-energy' bond equivalents (Livesey, 1993) represents energy not available for maintenance or growth. This term is similar to *net energy* used by Brown *et al* (1993) and Livesey (1991a), or *net energy for maintenance* used by Livesey (1995a) and describes the amount of food energy that is biologically useful in ATP equivalents (Warwick & Baines, 2000). Strictly speaking, this is not true because NME still includes the energy lost as heat produced during facultative and other types of thermogenesis, which is not used for ATP production.

Net energy (NE) is defined as ME less total heat losses from both obligatory and non-obligatory dietary thermogenesis, as well as thermogenesis due to effects of cold, drugs or hormones (Warwick & Baines, 2000). It is the energy equivalent of the portion of metabolisable energy that is completely useful to the body for maintenance and growth purposes. NE is a more precise estimate of the amount of energy available for ATP production than NME.

1.3.2 Energy requirement of the body

Evidence is emerging to suggest that the prevalence of obesity and the overweight condition is increasing worldwide at an alarming rate. New Zealand is no exception. In 2002/3 one in

three adults was overweight (excludes obese) and one in five adults was obese (Ministry of Health, 2003). Therefore, knowledge of energy requirements throughout the life cycle is essential to the promotion of optimal health. The World Health Organization (WHO, 1985) has defined the criteria for energy requirements as follows: “The energy requirement of an individual is the level of energy intake from food that will balance energy expenditure when the individual has a body size and composition, and level of physical activity, consistent with long-term good health; and that will allow for the maintenance of economically necessary and socially desirable physical activity. In children and pregnant or lactating women the energy requirement includes the energy needs associated with the deposition of tissues or the secretion of milk at rates consistent with good health.” For healthy individuals and with food being freely available, their energy intakes, energy expenditures and body stores should fit the following equation:

$$\text{Energy intake} = \text{Energy expenditure} \pm \text{Changes in body stores}$$

If an individual’s weight remains stable, then, over a period of time, energy intake must equal expenditure. In the past, the measurement of energy intake served as an important tool from which recommendations for energy requirements for all age groups were derived (WHO, 1985). However, since the advent of the doubly labelled water (DLW) technique, WHO has stated: “As a matter of principle we believe the estimates of energy requirements should, as far as possible, be based on estimates of energy expenditure” (WHO, 1985).

Total energy expenditure (TEE) includes the energy expended at rest, that expended in physical activity, and the thermic effect of food (Hildreth & Johnson, 1995). Unless an individual is very active, the major component of energy expenditure is the Basal Metabolic Rate (BMR). Basal metabolism represents the amount of energy required to maintain life-sustaining activities (breathing, circulation, heart beat, secretion of hormones) for a specific period of time. Basal metabolic rate is the rate at which the body spends energy to keep all these life-supporting processes going. BMR is usually measured in the morning upon awakening, before any physical activity and 12 to 18 hours after the last meal. Many scientists, however, prefer to use a more practical measurement called *resting energy expenditure* (REE). Resting energy expenditure is the energy a person expends in a normal life situation while at rest, and it includes some energy the body uses following meals and exercises. In general, BMR and REE differ by less than 10%, and the two terms are often used interchangeably. REE accounts for approximately 60% to 75% of our total energy expenditure (similar percentages to those of BMR). Two methods are used to measure REE

or BMR. One consists of human subjects residing in a chamber (known as a direct calorimeter), in which all the heat evolved can be measured. The second method, indirect calorimetry, uses a calorimeter to measure the amount of oxygen consumed. It is based on the fact that when an organic substance is completely combusted either in a calorimeter or in the human body, oxygen is consumed in amounts directly related to the energy liberated as heat. However, it is still not feasible to measure TEE, or even REE completely routinely. Whenever information about the energy requirements of a person is needed, practitioners still rely heavily on mathematical formulas that have been developed based on many hundreds of observations on people in many countries. In this study we use the equations published by WHO (1985) (**Table 1.4**) for predicting REE. These equations take into account age, sex, and weight, but ignore height, which was found not to affect the precision of prediction appreciably.

Table 1.4. Equations for predicting Resting Energy Expenditure (REE) in man from body weight (Adapted from NRC, 1990).

Sex and age range (years)	Equation to drive REE ^a (kcal/day)	Correlation coefficient ^b (r)	Standard deviation ^c (sd)
Male			
0-3	(60.9 × wt ^d) – 54	0.97	53
3-10	(22.7 × wt) + 495	0.86	62
10-18	(17.5 × wt) + 651	0.90	100
18-30	(15.3 × wt) + 679	0.65	151
30-60	(11.6 × wt) + 879	0.60	164
>60	(13.5 × wt) + 487	0.79	148
Female			
0-3	(61.0 × wt) – 51	0.97	61
3-10	(22.5 × wt) + 499	0.85	63
10-18	(12.2 × wt) + 746	0.75	117
18-30	(14.7 × wt) + 496	0.72	121
30-60	(8.7 × wt) + 829	0.70	108
>60	(10.5 × wt) + 596	0.74	108

^a These equations were derived from BMR data (WHO, 1985).

^b r: Correlation coefficient of reported BMRs and predicted values.

^c sd: Standard deviation of the differences between actual and computed values.

^d wt: Weight of person in kilograms.

The second largest component of energy expenditure after REE (or BMR) is physical activity. It varies the most, and may range from as little as 10% in the bedridden person to as much as 50% of total energy expenditure in the athlete. The amount of energy expended depends on the intensity and duration of activity. The energy costs of many different types

of work and activity have been measured (Durnin & Passmore, 1967; NRC, 1990; Shetty *et al.*, 1996) and expressed as multiples of REE, and an average daily ‘activity factor’ can be calculated based on these data and the time engaged in such activities. **Table 1.5** lists general activity factors associated with a range of activity patterns. The energy requirement for individuals, therefore, can be estimated by using WHO equations for calculating REE, and multiplying REE with an appropriate activity factor associated with an individual’s gender, age and daily activity level.

Table 1.5. Factors for calculating daily energy allowances at various levels of physical activity for men and women (ages 19 to 50) (Adapted from NRC, 1990).

Level of activity	Gender	Activity factor (x REE) ^a	Energy expenditure (kcal/kg per day) ^b
Very light	Men	1.3	31
	Women	1.3	30
Light	Men	1.6	38
	Women	1.5	35
Moderate	Men	1.7	41
	Women	1.6	37
Heavy	Men	2.1	50
	Women	1.9	44
Exceptional	Men	2.4	58
	Women	2.2	51

^a Based on examples presented by WHO (1985).

^b REE was computed from formulas in Table 1.4 and is the average of values for median weights of persons ages 19 to 24 and 25 to 74 years: males, 24.0 kcal/kg; females, 23.3 kcal/kg.

The third component of energy output is associated with the consumption of food. It is known as the thermic effect of food (TEF), or diet-induced thermogenesis, and accounts for approximately 10% of TEE (Poehlman & Horton, 1998). TEF can be separated into obligatory and facultative (or adaptive) subcomponents. Obligatory thermogenesis is the energy required to digest, absorb, and metabolise nutrients. It includes the synthesis and storage of protein, fat and carbohydrate. Adaptive or facultative thermogenesis is the ‘excess’ energy expended above the obligatory thermogenesis. It includes energy used to adapt to coldness, extreme changes in calorific intake, physical and emotional trauma, and is variable among people.

Overall, the total energy requirement of an individual can be estimated according to his or her total energy expenditure. Based on the energy requirement data published by WHO (1985), a general guideline on the daily energy intake of populations has been published by the National Research Council (NRC, 1990). The recommended energy allowances for adults with a light-to-moderate activity level are 2900 kcal/day for men of the standard body weight (~74kg), and 2200 kcal/day for women of the standard body weight (60-63 kg). For persons beyond age 50 the energy allowance is 2300 kcal/day for men, and 1900 kcal/day for women. The energy requirements for persons aged 75 or older are likely to be somewhat less as a result of reduced body size, REE, and activity. However, it must be considered that energy expenditure is affected by many variables, including age, sex, body size and composition, genetic factors, energy intake, physiological conditions, and ambient temperature. Even for those individuals following the same occupation and having similar physical characteristics, their energy requirements can be different. Any guideline only represents the average needs of individuals. For long-term health benefits, one must follow the principle of energy balance: when an individual's energy intake equals his/her energy expenditure their body weight should be constant. It is anticipated that, as much as possible, future estimation of energy intake will be based on actual measurements of energy expenditure.

1.3.3 Metabolism of energy in the human

Energy supplied in the diet is mostly in the form of complex macromolecules of carbohydrate, protein, fat (and sometimes alcohol), which with the exception of alcohol cannot be utilised by the body until they have been broken down into smaller molecules: monosaccharides, free fatty acids (FFA) and amino acids (AA). Some of the energy released during the process of digestion, absorption and metabolism of nutrients is captured within the coenzyme, adenosine triphosphate (ATP), a fuel for all energy-requiring processes in the body, but some escapes as heat that serves to assist to maintain body temperature. There is a range of energy-rich phosphorylated intermediates in the flow of energy utilisation within a cell, but ATP forms a link between them and, for simplicity, we may think of food energy as being converted exclusively into ATP energy. **Figure 1.4** gives an overview of the energy cycle in the body.

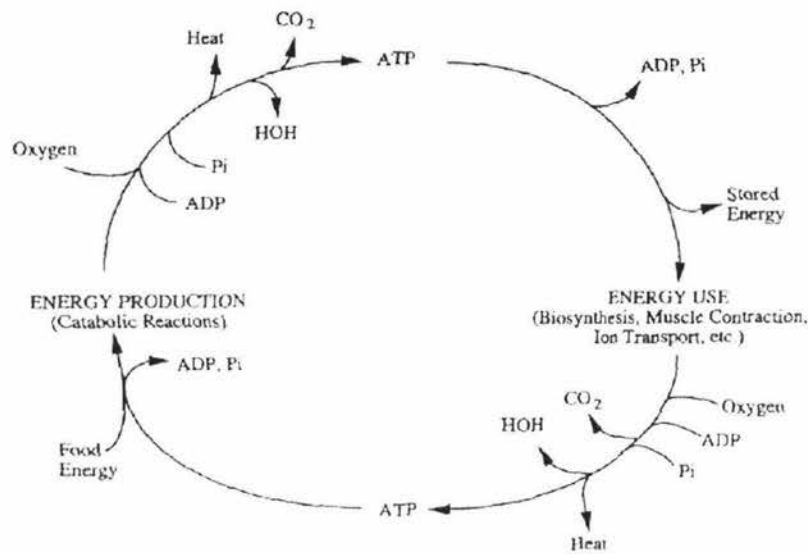


Figure 1.4. Overview of energy gains and losses in the body with ATP as the medium of exchange (Adapted from Berdanier, 2000).

The digestion of food begins in the mouth. In the mouth food is broken down into smaller pieces by mastication and mixed with saliva containing amylase by chewing. Starch is hydrolysed into simpler carbohydrate intermediary forms such as dextrin and maltose. Fat hydrolysis begins with lingual lipase secreted by the tongue and continues with gastric lipase in the stomach. Due to the complex structure of protein, protein hydrolysis does not begin until the gastric protease pepsin becomes activated in the stomach with its protein chain uncoiling. Pepsin breaks protein into polypeptides. Digestion continues in the small intestine: intestinal enzymes and specific pancreatic amylase works on starch intermediary products to break them down to monosaccharides. Bile excreted from the gallbladder emulsifies the fats, and pancreatic lipase breaks triglycerides into fatty acids, monoglycerides, and glycerol molecules. Pancreatic and intestinal proteases continue to hydrolyse the polypeptides into smaller peptides, then peptidases released from the cells of the brush border of the intestinal walls complete the hydrolyses of protein into absorbable units of individual amino acids and di/tripeptides. In the meantime, enzymes specific for disaccharides e.g. lactase for lactose, sucrase for sucrose, are also secreted by the small intestine's brush border cells, and they hydrolyse the disaccharides into monosaccharides. These monosaccharides are then absorbed, transported via the portal blood circulatory system to the liver, where fructose and galactose are converted into glucose. Aided by bile

salts, fatty acids, monoglycerides, and cholesterol move through the membrane wall, where fatty acids and glycerol combine back into triglycerides. These triglycerides are then incorporated into chylomicrons, which contain fats and cholesterol coated with protein, and travel through the lymph system to the blood circulatory system towards the hepatic portal system and liver. Some glycerol and any short- and medium-chain fatty acids are absorbed directly through the blood capillaries to the portal vein and liver. In the blood, triglycerides in the chylomicrons are broken down into fatty acids and glycerol by lipoprotein lipase, and are taken up by muscle cells, adipose cells and other cells in the vicinity. Di- and tri-peptides are broken down intracellularly and are transported mainly as free amino acids into the portal blood.

Once the large dietary molecules are broken down into smaller molecules and these circulate in the blood stream, the tissues can utilise them as fuel directly through catabolism. Glucose releases energy and is converted to carbon dioxide and water through the three processes of glycolysis, citric acid cycle, and oxidative phosphorylation. Through glycolysis, a glucose molecule produces pyruvic acid and ATP, oxygen is not needed and it provides energy for short, intense exertion. Pyruvic acid produced during anaerobic glycolysis reacts with coenzyme A (CoA) through an aerobic pathway via the TCA cycle; the energy process continues to produce additional ATP and carbon dioxide. Finally hydrogen is released as water through oxidative phosphorylation and more energy is captured as ATP. Overall, oxidation of 1mol of glucose, under aerobic conditions, leads to the formation of 36mol ATP. If oxygen is limited, pyruvic acid is reduced to lactate and the latter is taken up by the liver and converted back into glucose. Only two molecules of ATP are produced when one molecule of glucose is converted to lactate. The metabolism of free fatty acids (FFA) is dependent on oxidations to produce an activated two-carbon residue, acetyl CoA, which is then processed by the citric acid cycle to release energy ATP. Because of their structure, many fatty acids are able to form more ATP units than glucose and therefore provide more energy. For example, one molecule of palmitic acid can yield 129 molecules of ATP, of which 96 arise in the oxidation of eight molecules of acetyl-CoA; the corresponding figures for other fatty acids differ slightly and depend on the chain length and degree of unsaturation of each acid. Glycerol enters glycolysis by being transformed into pyruvic acid and is used the same way as glucose for energy. Amino acids (AA) after deamination are also oxidised in the cycle, but the intermediary metabolism and the point of entry into the cycle of individual acids varies greatly. The energy released from protein

hydrolysis and oxidation depends on the AA composition of the protein and about 30 percent of protein energy is excreted as urea. There is an energy cost in synthesising and excreting urea. **Table 1.6** shows how a diet providing 10.8 MJ might generate 142 moles of ATP. The energy equivalence of ATP is assumed as 33 kJ per mol. In this case about 4.5 MJ or 44 per cent of the dietary energy is converted into ATP energy, the remainder escapes as heat.

Table 1.6. Estimation of daily ATP production from a western diet (adapted from Passmore & Eastwood, 1986).

Food eaten	Amount		ME		ATP		
	g	mmol	kJ/g	Total kJ	ATP/mol	ATP/diet	Total ATP
CHO as glucose (MWt 180)	300	1667	16	4800	36		60.0
Fat as TG (MWt 861.5)	100	116	37	3700			
Glycerol (MWt 92)	10.7	116			20	2.3	
Sat. FA as palmitic (MWt 254)	22.1	87			129	11.2	
Monounsatur. FA as oleic (MWt 282)	49.1	174			144	25.1	
Polyunsatur. FA as linoleic (MWt 280)	24.4	87			142	12.4	51.0
Protein as AA (MWt 110)	80	727	17	1360			
Deamination to urea						1.4	
as glucose		288				10.4	
as 3-hydroxybutyric acid		145				3.7	
as acetoacetate		61				1.4	16.9
Alcohol as ethyl alcohol (Mwt 46)	35	761	29	1020	18		13.7
Total				10800			141.6

The production of ATP does not proceed at a constant rate but varies in response to the demand for energy by the cells. If more energy is consumed than is needed for immediate energy needs, extra carbohydrate is stored as glycogen in the liver and muscle. Excess fatty acids are reformed as triglyceride to be deposited in adipose cells. The energy cost of storing dietary fat as triglyceride is minimal. On the other hand, glucose can be used to make the stored triglycerides, but the process is quite inefficient: 49 ATP are used to make one molecule of tripalmitin from glucose compared to 12 ATP required for converting dietary to stored triglycerides (Blaxter, 1989; Passmore & Eastwood, 1986). Fats are denser energetically than carbohydrates. On a per gram basis fat provides 9 kcal (37.6kJ) and carbohydrate 4 kcal (16.7kJ) metabolisable energy, respectively. Using protein for energy is

even more costly and less efficient. When dietary energy is deficient energy consumed is used immediately regardless of its source. Then stored glycogen is used through glycolysis followed by body fat stored in adipose cells. Body protein is used for energy production (gluconeogenesis) as a last resort.

1.3.4 Current status of energy expression

As discussed above, energy is gained from oxidation of food-derived fats, carbohydrates, and proteins, and is transmitted via ATP (and other high-energy transfer compounds) to a variety of essential macro- and micromolecules in the body, which in turn, are used to maintain life processes. However, the amount of energy available from food and the efficiency of energy metabolism in the whole body is influenced by numerous factors, such as the composition of the food, the status of nutrition (overfed or underfed), the extent of digestion and absorption of food, the extent of fermentation in the colon, the oxidation by metabolic processes, drug intakes, hormone secretions, environmental temperature and so on (Allison & Senti, 1983; Warwick & Baines, 2000). For example, dietary fibre (unlike starch) passes through the digestive tract largely unchanged to the large intestine, but it influences events at all levels of the gastrointestinal tract. An increased intake of dietary fibre promotes a feeling of satiety (Heaton, 1986), reduces the rate of gastric emptying of nutrients (Leeds *et al.*, 1981), which may slow glucose absorption (Torsdottir *et al.*, 1984) and reduce energy intake. Sugar alcohols are often considered poorly or nondigestible carbohydrates compared to glucose, but the digestion of sugar alcohols does occur. The quantity of energy available from each sugar alcohol depends on its fate in the human body. Research data also show that the intake of dietary energy influences nitrogen balance, at surplus as well as at deficient energy intake (Göranzon *et al.*, 1983). Therefore, a valid expression of food energy values, especially for foods or diets that are high in protein, fermentable carbohydrates or sugar alcohols, is essential in the development of low-calorie food and weight control diets, in assessment of dietary intakes of groups and populations and in maintaining energy balance.

Metabolizable energy (ME) has traditionally been used to estimate how much food energy is available to the body in relation to energy requirements. It has been defined as ‘food

energy available for heat production (= energy expenditure) and body gains' (Atwater & Bryant, 1900), and more recently as 'the amount of energy available for total (whole body) heat production at nitrogen and energy balance' (Livesey, 2001). The most widely utilized system for estimating the metabolizable energy value of foods is based on the work of Atwater carried out at the turn of the twentieth century (Atwater & Bryant, 1900).

A NME system has recently been proposed by Livesey and his coworkers (2000, 2001) to replace the traditional ME system of food evaluation. Net metabolizable energy (NME) represents food energy available to the body after the heat produced during fermentation and obligatory thermogenesis has been deducted from ME (Livesey, 1993). It describes the amount of food energy that is biologically useful in ATP equivalents and is used to derive energy factors for sugar alcohols, polyols and polydextrose (FASEB, 1994). However, NME does not deduct the heat produced during facultative and other types of thermogenesis, which is energy not available for net ATP production. From this view point, net energy (NE) is perhaps a more valid expression of the amount of energy available for ATP production than NME. But in reality, NE values are difficult to obtain for individual foods or food components because various energy-yielding components are used with different efficiencies, depending on the physiological functions (e.g. maintenance, growth or muscle activity). It is impossible to assign a single net energy value to a food for all of these functions. Current estimates of energy requirement for healthy people are also derived from measures of energy expenditure and are expressed in terms equivalent to the ME (WHO, 1985; NRC, 1990), therefore, at the present time the metabolizable energy system is still preferable when evaluating the caloric value of foods eaten by humans and for food labelling and regulatory purposes (Warwick & Baines, 2000).

The metabolizable energy (ME) value of a food represents the amount of energy available for total heat production (HE) and for body gains such as growth and fat deposition (RE), i.e.

$$ME = HE + RE.$$

When there is no change in the energy content of the body (i.e. subject is in energy balance: RE = 0), then ME = HE, indicating that the energy available from food is used only for basal metabolism, muscular activity, and the heat increment of maintenance (H_iE). H_iE is referred to as the thermic effect of food, or diet-induced thermogenesis. Energy losses in hair, skin, nails, and secretions other than urine and faeces are generally regarded as being

small and negligible. Gaseous losses are usually less than 1% of ingested ME (Blaxter, 1989; Poppitt *et al.*, 1996). Thus, it is generally acceptable that ME be directly measured in balance experiments as:

$$\text{ME} = \text{Gross food energy} - \text{faecal energy} - \text{urinary energy}; \text{ (Buskirk \& Mendez, 1980).}$$

1.4 Determination of the metabolizable energy value of foods

An accurate evaluation of the energy value of foods is essential for sound nutritional management. The amount of dietary energy available for maintenance and performance in humans (i.e. metabolizable energy) can be determined experimentally by conducting human balance studies and directly measuring the losses of energy in faeces and urine. The measurement is not technically difficult, but is time-consuming, expensive and, most important of all, it places quite severe restrictions on the activities and mode of life of the person eating the diet. It is also rather impractical as there is a wide variety of foods eaten by humans. Therefore, prediction models have been developed to estimate metabolizable energy values based on food chemical analytical values. Models fall into two distinct groups: factorial and empirical (Livesey, 1995b). Factorial models are based on analysis of each of the energy-yielding substrates (e.g. protein, fat, carbohydrate, and dietary fibre), with each component being assigned an energy value based on experimental determination of apparent digestibility regardless of the food in which it is found (Atwater, 1910; Miller & Payne, 1959). Empirical models are based on the analysis of the gross energy value of foods or diets and predictors of energy excretion based on the intake of unavailable carbohydrate (UC) and nitrogen. Such formulae are usually obtained by regression analysis relating experimentally determined dietary energy values to compositional determinants such as gross energy value, nitrogen content, UC or dietary fibre content. From a scientific point of view, empirical models are preferable to factorial models (Livesey, 1995b), especially for fibre-rich diets; however, the ingrained inertia of factorial models and the complexity and limitation of empirical models has precluded the use of empirical models in food labelling regulations and food tables.

1.4.1 Factorial systems

Atwater's general factors

The classic investigations of Professor W O Atwater and his associates at the Storrs (Connecticut) Agricultural Experiment Station, 100 years ago provided the basis of the factorial system used today for calculating the metabolizable energy values of foods and diets. In general, the Atwater system for estimating the “physiological” energy of a specific food consists of a series of factors:

- a) proximate composition of a food
- b) values for the heats of combustion of protein, fat, and carbohydrate
- c) coefficients of digestibility
- d) energy lost in urine

In a proximate analysis, fat or lipid is determined gravimetrically after solvent extraction by procedures dependent on the nature of the food. Protein is calculated by applying a conversion factor to the measured nitrogen content, and carbohydrate is calculated by difference (subtracting the percentage of protein, fat, moisture, and ash from 100). However, the methods used for determining the energy-yielding components in a food at the time the Atwater system was devised were not entirely satisfactory. It was also realized, as early as 1885, that the heat of combustion values for pure protein, neutral fat, and pure carbohydrate might not be applicable to each of the energy-yielding components in foods (Rubner, 1885). Based on the determined heat of combustion of fats and oils of different origin, Atwater considered that the heat of combustion factors determined for triglycerides were best suited to apply to the fat content of food, which was based on the assumption that the error resulting from the use of the higher heat combustion factors would in some measure offset the error resulting from the incomplete extraction of fat in the determination of the fat content of the food (Atwater & Bryant, 1900). Atwater also considered 4.2 kcal per gram the suitable heat of combustion factor to use for carbohydrates in cereals and vegetables; 4.0 kcal for carbohydrates in fruits; and 3.9 kcal for carbohydrates in all foods of animal origin. By accounting for the variation in the total nitrogen content of different kinds of protein and the proportion of protein and non-protein nitrogenous material, Atwater assigned protein a series of heat of combustion factors depending on the protein origin. Then Atwater applied these combustion factors to 276 food samples including foods of animal origin as well as a variety of plant products to assess their accuracy. Bomb

calorimetry measurements confirmed that the calculated and determined results were in good agreement. Therefore, Atwater concluded that the gross energy of a food or mixed diet could be predicted by applying derived heat of combustion factors to each of the energy-yielding components in a food and calculating the sum. The average heat of combustion factors were 5.65, 9.40, and 4.15 kcal per gram for protein, fat, and carbohydrate (by difference) in the mixed diets, respectively (Atwater & Bryant, 1900).

To estimate the coefficients of digestibility for protein, fat, and carbohydrate components of individual foods, Atwater also examined data from digestion experiments involving single foods or very simple mixed diets and assigned a set of digestibility coefficients to protein, fat and carbohydrate in major food groups (Atwater & Bryant, 1900). Then Atwater tested these coefficients in experiments when ordinary mixed diets were eaten. Based on the experimental data obtained from subjects consuming these diets, coefficients were altered slightly to bring calculated values and experimental results into close agreement. The resulting average coefficients of apparent digestibility (Atwater used the term availability) for the nutrients in a mixed diet were 92, 95 and 97% for protein, fat, and carbohydrate, respectively. When these coefficients were applied to data in 93 digestion experiments on ordinary mixed diets very good agreement was found between calculated values and the results of actual determination. From this, Atwater concluded that for common mixed diets the average digestibility coefficients were accurate enough, but he pointed out that the average digestibility coefficients might not be applicable under all circumstances and might not apply to all foods in one class.

To correct for the loss of incompletely oxidized nitrogen from the body, Atwater determined the ratio of nitrogen in the urine to the heat of combustion of the urine in 46 digestion experiments. An average value of 7.9 kcal per gram of urinary nitrogen, which corresponds to 1.25 kcal per gram of protein absorbed (ingested less digestive loss), was assigned as the energy loss due to incomplete metabolism of protein (Atwater & Bryant, 1900). In the case of digestible meat protein, for example, 1.25 would be deducted from the heat of combustion per gram (5.65 kcal) to correct for the heat of combustion of the unoxidized products in the urine. The resultant fuel value of 4.40 kcal would be applied to each gram of protein available as a source of fuel. However, several questions have been raised concerning the advisability of applying the calorie-nitrogen ratio (7.9 kcal/N) published by Atwater (Atwater & Bryant, 1900), to energy calculations where dietary

conditions may be different. Experimental data show that the ratio is affected by the proportion of protein, fat, and carbohydrate in the diet (Lusk, 1928). For example, experimental data show that the urinary calorie-nitrogen ratio may vary from 6.44 to 10.36. Also, Atwater's ratio of 7.9 kcal/N, was based on the assumption that the experimental subjects were in nitrogen balance and that all of the nonmetabolized N was recovered in the urine. There was no allowance to correct for nitrogen balance. Nevertheless, the amount of energy lost in the urine, on the whole, is small compared with the gross energy of the food eaten, and detailed data for the digestibility and utilization of various nitrogenous compounds are not available, Atwater's average calorie-nitrogen ratio (7.9 kcal per gram of urinary nitrogen, 1.25 kcal per gram of digested protein), is still in use today to correct energy loss from the incomplete metabolism of protein.

Based on the average heat of combustion factors corrected for urinary N energy loss (4.40, 9.40, and 4.15 kcal per gram protein, fat, and carbohydrate by difference) in a mixed diet, and the average coefficients of apparent digestibility of nutrients in a diet (92, 95 and 97% for protein, fat, and carbohydrate, respectively) Atwater and his co-workers produced a set of factors (4.05, 8.93, and 4.03 kcal per gram protein, fat, and carbohydrate in a mixed diet) to estimate available energy values of foods. These factors have been rounded to 4, 9, and 4 kcal/g (Atwater, 1910) and have become known as 'Atwater's general factors'. Atwater's general factors also include a factor for alcohol with a rounded value of 7.0 kcal/g (29 kJ/g) or an unrounded value of 6.9 kcal/g (Atwater & Benedict, 1902). Atwater's general factors have gained widespread acceptance for calculating the metabolizable energy values of foods, not only for a mixed diet as Atwater had originally intended but also for individual foods (Merrill & Watt, 1973; Allison & Senti, 1983). Atwater's general factors, however, didn't correct for the energy cost related to the synthesis and excretion of urea in the body, which requires a considerable amount of ATP energy. When protein is the major energy source of the diet, such as in high protein, low Carb weight-loss diets, ME values estimated using Atwater's general factors would be questionable. In the meantime the assumption that the energy factor of each component in a food is fixed and not affected by the proportions of other components is also questionable, especially for those diets very high in fibre, or that may contain novel ingredients. Such diets and foods are becoming more common, with a modern emphasis on dietary intervention to achieve body weight loss.

Atwater's specific factors

It has been recognized that the use of Atwater's general factors for analysis of specific foods may introduce significant errors in estimating the energy value. When Atwater proposed his individual food group factors and his general factors for estimating the energy value of mixed diets, data for a number of foods had been accumulated to make possible revisions later. Since then, more data have accumulated in the scientific literature. Based on re-examination of the Atwater system, Merrill and Watt (1955) introduced the Atwater specific factor system, also known as Merrill and Watt (1973) factors. They integrated the results of 50 years of research and derived different factors for proteins, fats, and carbohydrates, depending on the foods in which they are found. Therefore, the metabolizable energy value of a food or whole diet can be calculated as a sum of the available energy of each item:

$$ME_{M\&W} = (w_1P+x_1F+y_1C)_{food1} + (w_2P+x_2F+y_2C)_{food2} \dots + (w_nP+x_nF+y_nC)_{foodn}$$

Where w , x , and y represent the specific calorie-conversion factors for protein (P), fat (F), and carbohydrate (C), respectively, and 1, 2, n represent the food or ingredient.

Atwater's specific factors emphasized that the heat of combustion and coefficients of digestibility are different among different proteins, fats and carbohydrates. They generate a set of tables with substantial variability in the energy factors to be applied to various foods (Merrill & Watt, 1973). For example, the energy factor of protein is 4.36 kcal per gram for egg compared to 3.82 kcal per gram for white rice. Merrill and Watt (1973) compared the energy values for different representative foods and food groups derived using specific factors with those derived using Atwater general factors. On average the special values were about 5 percent higher than the general values. There were several foods (for example snap beans, cabbage and lemon) for which the differences ranged from 20 to 38 percent. When these foods were not included, the average difference between general and specific factor values was 2 percent. More comparisons have been published since the original validation of the specific factor system and it can be concluded that the Atwater specific-factor system appears to predict availability of energy even from diets containing large amounts of unavailable carbohydrate with no overall bias (Livesey, 1990). Generally, the Atwater specific-factor system appears to be superior to the original Atwater general system. However, it is cumbersome and complex to use. Recent research data from Brown *et al* (1998) show that the specific-factor system was without bias for a low fibre diet, but underestimated (5.3 and 3.7%, respectively) available energy for high NSP maintenance

diets. For high fibre Westernized diets, the specific factor system is often accurate to within 6% of the determined metabolizable energy value (Calloway *et al.*, 1978; Göransson *et al.*, 1983; Miles *et al.*, 1988; Brown *et al.*, 1998). Therefore, the Atwater specific factor system may not be accurate when be applied to ‘extreme’ diets, such as specialized low-calorie foods, where small differences in ME need to be detected accurately. It also needs to be appreciated that in diets of ‘extreme’ composition such as those very high in dietary fibre, physiological energy values may not be additive for all foods.

British system

Although Atwater’s general factors are still used extensively, other countries have evolved slightly different systems for calculating the ME value of mixed diets. In Britain, McCance and his associates have given special attention to assessing the energy value of cereals (McCance & Glaser, 1948; McCance & Walsham, 1948). The consideration given to carbohydrate has been an important difference between the systems used for estimating energy values in Britain and American. The British or U.K. system measures the content of available carbohydrate directly (McCance & Widdowson, 1940; Paul & Southgate, 1978; Holland *et al.*, 1991). Glucose, fructose, sucrose, dextrans and starch are determined separately and their sum is given as ‘available carbohydrate’ expressed in terms of monosaccharide. A factor of 3.75 kcal/g is assigned to this available carbohydrate fraction. Following this procedure, the energy factor for starch is 4.13 kcal/g, because 100g of starch yield 110 g of monosaccharides on hydrolysis ($110 \times 3.75 / 100 = 4.13$). Disaccharides have a value of 3.94 kcal/g ($105 \times 3.75 / 100 = 3.94$) (Paul & Southgate, 1978; Holland *et al.*, 1991). In the British system, the calorific factor used for protein in early editions of the UK food tables was 4.1 kcal/g based on the work of Rubner (1885). The current edition of the UK food tables lists a factor of 4.0 kcal/g for the protein component, where the latter is calculated from nitrogen content, using specific factors where available (Paul & Southgate, 1978; Holland *et al.*, 1991). Ethyl alcohol, acetic acid, citric acid, lactic acid, and malic acid are all assigned specific calorific values in the British system (**Table 1.7**).

The British system considered that the ME of a diet could be calculated without specific inclusion of a value for ‘unavailable carbohydrate’. The scientific rationale for accepting a zero calorific value for ‘unavailable carbohydrate’ was that the energy obtained from dietary unavailable carbohydrate by humans (including action of the colonic microorganisms) was approximately equally balanced by additional losses of energy to

faeces in the form of fat and protein, which arose from an increased intake of the unavailable carbohydrate (Southgate & Durnin, 1970). However, results from recent experiments involving high-fibre diets have shown that dietary fibre can contribute substantially to the ME of a diet, but there are large variations among the determined energy values of unavailable carbohydrate (UC) ranging from well below zero (-5 kcal/g) to above zero (3 kcal/g) (Göranzon *et al.*, 1983; Miller & Judd, 1984; Göranzon & Forsum, 1987; Miles *et al.*, 1988; Livesey, 1990; Wisker *et al.*, 1992; 1996a & b; 1997). The calorific conversion factors devised in the British system were based on the diets containing ~10-30g unavailable carbohydrate, and the influence of unavailable carbohydrate on losses of protein and fat to faeces has been accounted for up to this level of intake (Southgate & Durnin, 1970). Therefore, by assigning a zero apparent digestible energy value for unavailable carbohydrate the British system tends to underestimate the energy value of diets, especially for diets containing high levels of unavailable carbohydrate, due to a double accounting of the influence of the ingestion of unavailable carbohydrate on the energy availability of diets (Livesey, 1990).

Table 1.7. Energy conversion factors used in MaCance and Widdowson's the Composition of Foods table (Holland *et al.*, 1991).

Food components	kcal/g	kJ/g
Protein	4	17
Fat	9	37
Available carbohydrate expressed as monosaccharide	3.75	16
Alcohol	7	29
Acetic acid	3.5	14.6
Malic acid	2.4	10.0
Lactic acid	3.6	15.1
Citric acid	2.4	10.3

New Zealand system

In the same manner as the British system, the New Zealand system measures carbohydrate directly. In the New Zealand food tables, the determined carbohydrate component represents the amount of available carbohydrate, which is measured directly and equals the sum of the individual mono- and disaccharides and starch, expressed as the weight of the carbohydrate. The energy factors used for calculating the metabolizable energy value of

foods from the energy-yielding food components are listed in **Table 1.8**. Energy factors of 4, 9, 4 kcal/g have been assigned to protein, fat and carbohydrate, respectively. Energy value can be expressed either in kilocalories (kcal) or in kilojoules (kJ) (1 kcal = 4.184 kJ). A zero energy factor has been assigned to dietary fibre in the New Zealand food composition table (Athar *et al.*, 2003). The protein content of food is based on total nitrogen multiplied by a factor related to the amino acid composition of the food. No attempt has been made to correct for non-protein nitrogen present in the food. Total fat is measured by standard gravimetric methods, and includes phospholipids and wax esters, as well as minor amounts of non-fatty material (AOAC, 2000). The fat value can vary depending on the analytical procedures used.

Table 1.8. Energy conversion factors used in New Zealand food composition tables (Athar *et al.*, 2003).

Food component	kcal/g	kJ/g
Protein	4.00	16.7
Fat	9.00	37.7
Available carbohydrate expressed as weight of CHO	4.00	16.7
Ethyl alcohol	7.00	29.3
Acetic acid	3.49	14.6
Malic acid	2.39	10.0
Lactic acid	3.62	15.1
Citric acid	2.47	10.3

Nutrition Information Panels (NIP) are required by law on the packaging of foods for sale in New Zealand and Australia and the Australia New Zealand Food Authority (ANZFA) has reviewed the scientific basis for energy factors used for nutritional labelling in Australia and New Zealand. In order to align with internationally recognised definitions of metabolizable energy, that used in Australia and New Zealand food standards for the purposes of food labelling is:

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

Where: GE is gross energy (as measured in bomb calorimetry)
 FE is energy lost in faeces
 UE is energy lost in urine
 GaE is energy lost in gases produced by fermentation in the large intestine
 SE is energy content of waste products lost from surface areas

This definition is completely compatible with energy expenditure measurement. For most conventional foods energy losses from gases and the body surface are minimal, and the calculated energy factors derived using this definition will be similar to those derived using Atwater's ME definition ($ME = GE - FE - UE$). **Table 1.9** lists the energy conversion factors used for NIP in New Zealand. The general factors for fat, protein, carbohydrate, and alcohol are based on Atwater's general factors. An energy factor of 8 kcal/g has been assigned to unavailable carbohydrate based on research data from the British Nutrition Foundation (BNF, 1990; ANZFA, 1999). The Prosky method (AOAC total dietary fibre method: 991.43) is recommended for fibre analysis in New Zealand for food labelling (AOAC, 2000). The energy factors for polyols, isomalt, polydextrose and organic acids were also reviewed. The values assigned for each component were based on a report from the Federation of American Societies for Experimental Biology prepared for the US Calorie Control Council in June 1994 (FASEB, 1994). It is obvious that the energy factor assigned to dietary fibre is different for the purposes of food labelling and food composition tables in New Zealand. The present study uses the energy factors given in the New Zealand food tables.

Table 1.9. Energy conversion factors used for Nutrition Information Panels in New Zealand (Athar et al., 2002).

Food component	Energy factor (kJ/g)
Protein	17
Fat	37
Carbohydrate (excluding unavailable carbohydrate)	17
Unavailable carbohydrate (including dietary fibre)	8
Alcohol	29
Erythritol	1
Glycerol	18
Isomalt	11
Lactitol	11
Maltitol	16
Mannitol	9
Organic acids	13
Polydextrose	5
Sorbitol *	14
Xylitol	14

* An average of a calculated range determined with or without ingestion of other foods.

Other factorial systems

Due to an increasing dietary fibre intake and controversy over the contribution of dietary fibre to the energy value of diets, the reliability of food energy assessment systems for higher fibre maintenance diets has been studied widely (Southgate & Durnin, 1970; Miller & Judd, 1984; Livesey, 1990; 1991a; 1991b; 1992; 1993; 1995a; 1995b; Wisker *et al.*, 1993; 1996a; 1996b; Barry *et al.*, 1995; Behall & Howe, 1995; Baer *et al.*, 1997). Modified factorial systems of food energy assessment, which take into account the variable dietary fibre content, have resulted FDA system, 1993 and Livesey's factors, 1990:

$$\text{FDA} \quad \text{ME (kJ)} = 16.7 P + 37.6 F + 16.7 (C - \text{isDF}) \quad (\text{FDA, 1993})$$

$$\text{Livesey} \quad \text{ME (kJ)} = 16.7 P + 37.6 F + 15.7 C_m + 8.4 U \quad (\text{Livesey, 1990})$$

Where: P (g) = dietary protein content
F (g) = dietary fat content
C (g) = Carbohydrate by difference
isDF = insoluble dietary fibre determined by following AOAC enzymatic-gravimetric method (AOAC, 2000)
C_m (g) = available carbohydrate determined directly and expressed as equivalent weight of monosaccharide
U (g) = unavailable carbohydrate, sum of non-starch polysaccharide and resistant starch

The FDA regards 16.7 kJ (4 kcal), 16.7 kJ (4 kcal) and 37.6 kJ (9kcal) as the energy available from 1 g carbohydrate, protein and total fat, respectively, based on the derivation of Atwater's calorie values outlined in USDA Handbook No. 74 (slightly revised in 1973). Carbohydrate is determined by difference. To compensate for the energy loss caused by an increased intake of unavailable carbohydrate, FDA recommended that carbohydrate should be calculated as carbohydrate by difference less the insoluble fibre content (FDA, 1993). This was based on the digestible energy value of dietary fibre in mixed diets being approximately 8.4 kJ/g (2 kcal/g) (Livesey, 1990; Livesey, 1991a). The bias of the FDA factors is considered small and would be zero when 50% of the unavailable carbohydrate ingested is soluble dietary fibre (BNF, 1990). However, it is uncertain that the dietary fibre in average US diet contains 50% soluble dietary fibre, and several studies have shown that solubility is not a good determinant of the energy value of dietary fibre in both humans and laboratory rats (Livesey, 1995c).

Livesey derived his factors (Livesey, 1990) after analysing the results of published data on 29 human diets containing unavailable carbohydrate from several sources and with intakes

ranging from 4 to 93 g/d. Livesey used the concept of partial digestibility, as described for dietary supplements by Kleiber (1975), to describe 'associative effect' on the losses of protein and fat to faeces due to increased unavailable carbohydrate intake and the energy contributed from unavailable carbohydrate intake. After examining the relationships among the apparent digestibilities of unavailable carbohydrate, intake and its partial indigestibility for energy, Livesey concluded that unavailable carbohydrate was the major dietary factor influencing digestible energy in the whole diet, as well as being the major factor influencing the digestibility of dietary gross energy. A caloric factor for unavailable carbohydrate about midway between the value implied in the Atwater system (16.7 kJ/g, or 4 kcal/g) and the British systems (0 kJ/g or 0 kcal/g) could be of practical use. Therefore, he adopted 8.4 kJ (2 kcal) per gram unavailable carbohydrate with the British system to create a new modified factorial system known as Livesey factors (or modified Atwater factor system). The energy factors 16.7 kJ (4 kcal), 37.7 kJ (9 kcal), 15.7 kJ (3.75 kcal), and 8.4 kJ (2kcal) were assigned per gram of protein, fat, available carbohydrate (determined directly and expressed in monosaccharide weight equivalents) and unavailable carbohydrate, respectively. The Livesey factors appear suitable for use in most circumstances.

1.4.2 Empirical Models

Unlike the factorial system, empirical models are based on gross energy data (Brown *et al.*, 1993; Livesey, 1995b), which can be determined accurately. Various empirical models have been developed since Levy *et al* (1958) first proposed an empirical model for estimating the ME value of diets based on the gross energy value (kcal/g, determined by bomb calorimetry) and total nitrogen content (g/kg) of the diet. **Table 1.10** lists several commonly used empirical models for predicting the ME value of diets.

The model developed by Levy *et al* (1958) was based on diets with a limited range of unavailable carbohydrate and has been shown to overestimate the ME values of diets that contain high amounts of unavailable carbohydrate (Livesey, 1990). Based on an average figure that 95% of food energy is digestible for a mixed diet (Atwater & Bryant, 1899), Miller & Payne (1959) put forward that metabolizable energy of human diets can be assessed by subtracting the energy losses for the indigestible portion in the food, and the loss associated with the incomplete oxidation of nitrogen in the body. The value of 31.4 kJ

(7.5 kcal) was suggested as the figure for urinary loss from 1 g of food nitrogen (Rubner, 1885; Atwater & Bryant, 1899). Miller & Payne's equation (1959) was derived from diets containing low-to-moderate quantities of unavailable carbohydrate. When it is applied to diets containing high amounts of unavailable carbohydrate it tends to overestimate the ME value of the diet, because the digestibility of food energy is probably less than 95% on foods high in unavailable carbohydrate (Livesey, 1990).

Table 1.10. Empirical models for predicting metabolizable energy values of diets (adapted from Livesey, 1995b).

Reference and year	Model	Notes
1958: Levy <i>et al</i>	$ME = 0.976E - 33.3N - 250$	Overestimates ME at high UC intake
1959: Miller & Payne	$ME = 0.95E - 31.4N$	Overestimates ME at high UC intake
1975: Southgate	$ME = 0.977E - 16.7UC - 27.6N$	Underestimates ME at high UC intake
1984: Miller & Judd	$ME = (0.95 - UC\%)E - 31.4N$	Underestimates ME at high UC intake
1990: Livesey	$ME = 0.978E - (17.2UC \times S) - 30N$	No bias, computation of S^* is complex
1991a: Livesey	$ME = 0.97E - 56UC^{0.6} - 30N$	No bias
1991a: Livesey	$ME = 0.96E - 8.4UC - 30N$	No bias
1993: FDA	$ME = E - 5.23P$	Always overestimates ME
1993: Brown <i>et al</i>	$NME = 0.96E - 10.5UC - 50N$	

ME: metabolizable energy (kJ); E: gross energy (kJ); N: dietary nitrogen (g); UC: unavailable carbohydrate (g); UC%: unavailable carbohydrate as a percentage of the dry weight of food; UC^{0.6}: (g^{0.6}); P: protein (g). $S = 1 / (2.6\alpha + 6.2\beta - 0.96)$ where α (a fraction) is the fermentability of the unavailable carbohydrate β (a fraction) is the proportion of gross energy intake contributed from unavailable carbohydrate.

The Southgate model (1975) was derived from data on nine diets, whereby unavailable carbohydrate content ranged from 6 to 32 g daily. It applied an apparent digestibility coefficient of 0.977 for dietary gross energy, and it was shown experimentally that the ingestion of more unavailable carbohydrate did not, on average, alter the apparent digestibility of dietary gross energy (Southgate & Durnin, 1970; Southgate, 1975). A value of 16.7 kJ (4 kcal) per gram ingested unavailable carbohydrate was subtracted, to balance the gross energy intake from unavailable carbohydrate, that was included in the term of 0.977E. Because the effect of unavailable carbohydrate on the losses of protein and fat to faeces diminishes with its increased intake level, the Southgate model tends to overestimate energy losses from faeces, and therefore underestimate the ME value of diets containing high levels of unavailable carbohydrate (Livesey, 1990).

To address the shortcomings of the Southgate and Miller & Payne models, Miller & Judd (1984) proposed an equation based on the assumption that the efficiency of digestion of all the energy containing constituents of the diet was influenced by dietary fibre content. For diets containing no fibre Miller & Judd's equation would be reduced to Miller & Payne's equation. The Miller & Judd equation was derived from diets containing barley, which may not be representative of westernized mixed diets. Livesey tested the latter equation using 17 diets, and demonstrated that the equation underestimates the amount of available food energy, increasingly with increased unavailable carbohydrate intake (Livesey, 1990).

Based on 17 diets with varying levels of unavailable carbohydrate, Livesey derived an equation ($ME = 0.978E - (17.2UC \times S) - 30N$) containing a term called 'S', for predicting the ME value of mixed diets (Livesey, 1990). Term 'S' indicates faecal energy excretion based on the intake and fermentability of unavailable carbohydrate. In the meantime Livesey derived another equation ($ME = 0.97E - 56UC^{0.6} - 30N$) based on data for 43 diets widely ranging in unavailable carbohydrate (2-93g UC daily), and used unavailable carbohydrate as a power term ($g^{0.6}$) (Livesey, 1991a). The response of faecal energy excretion to unavailable carbohydrate intake is curvilinear for both models, therefore the sum of the metabolizable energy calculated based on individual foods cannot be equal to the metabolizable energy calculated for the whole diet. Although these models seem to be good predictors of dietary energy availability, they are impractical for general use (Livesey, 1995b). In order to simplify the predictive procedure, Livesey also derived a linear equation ($ME = 0.96E - 8.4UC - 30N$) based on the same 43 diets (Livesey, 1991a). In this model, faecal energy losses were predicted as a proportion of the gross energy intake (0.04E), therefore the energy availability of the diet would be 0.96E. Urinary energy losses were assumed as 30 kJ (7 kcal) per gram nitrogen intake, and 8.4 kJ (2 kcal) as the energy value for unavailable carbohydrate. The difference between predicted and determined ME values for mixed diets was generally less than 2% and often not >1% (Livesey, 1991a). It seems to be the best model for predicting the ME value of a mixed diet derived to date.

The model proposed by the Food and Drug Administration (FDA) (1993) only accounted for the gross energy intake (kJ) and energy losses from urine. The factor 5.23 was Atwater's correction for the urinary energy associated with digestible protein intake (not total protein intake) (Merrill & Watt, 1973). Therefore, the FDA model would always overestimate the energy availability of diets, especially for high unavailable carbohydrate intakes.

The last equation (Brown *et al.*, 1993) listed in Table 1.10 was also derived based on Livesey's observations for 43 diets with unavailable carbohydrate daily intake ranging from 2 to 93 g. Strictly speaking it goes further than predicting ME, it makes deductions for the energy excretion as combustible gases (hydrogen and methane), the heat losses associated with fermentation of unavailable carbohydrate, and the cost of excretion of ammonia nitrogen as urea. It is outside the range of the presently reported study and won't be discussed further.

1.5 Analysis of energy-yielding components

Analytical methodology and proper definitions for energy-yielding components in food play an important role in establishing accurate methods for calculating the available energy value of diets and foods. Rubner, as early as 1885, had pointed out that methods for determining the three major food energy-yielding components - carbohydrate, fat and protein, were not entirely satisfactory and might limit the application of data listed in food composition tables. Since then innumerable improvements in methods and techniques for separating and determining these food components have been made. A brief outline of the development of analytical methods and changes in relevant definitions is given.

1.5.1 Analytical methods for carbohydrates in foods

Determining carbohydrate 'by difference' has been the approach used in the Atwater system since the beginning of the twentieth century (Atwater & Bryant, 1899). The carbohydrate content is determined as the difference between 100 and the sum of the percentage of water, protein, fat, and ash. Total carbohydrate by difference includes fibre, as well as some components that are not, strictly speaking, carbohydrate, e.g. organic acids (Merrill & Watt, 1973). Determining carbohydrate 'by difference' provides the Atwater system with certain advantages in predicting ME values by compensating for the errors that may arise from protein analysis, by inverse changes in the determined amount of carbohydrate, since both components are assigned similar physiological energy values. Similarly, errors in fat analysis are also partially compensated for. It is recognised that this compensating feature is an asset of the Atwater system, which confers it with certain

robustness. Further, calculating carbohydrate ‘by difference’ ensures that the total food dry matter and gross energy is apportioned to nutrients. A current modification in the United States system allows carbohydrate to be calculated by difference less insoluble fibre (FDA, 1993). Dietary fibre is directly determined by the AOAC method (AOAC, 2000). Total carbohydrate can also be calculated from the sum of the weights of individual carbohydrates and fibre after each has been analysed directly.

Unlike the situation in the United States, in the UK the carbohydrate fraction is divided into two main groups for purposes of assigning energy factors: available carbohydrate and unavailable carbohydrate. McCance and Lawrence (1929) devised analytical procedures to measure the content of available carbohydrate and unavailable carbohydrate in foods to obtain information on carbohydrate needs for the treatment of diabetics. Southgate (1969a & b) developed those procedures further. Available carbohydrate was defined as the carbohydrate fraction which can be digested and absorbed by humans and that is glucogenic. Available carbohydrates include starch and soluble sugars, and can be measured in two different ways: estimated by difference or analysed directly. In either way, available carbohydrate can be expressed as the weight of the carbohydrate or as monosaccharide equivalents.

To calculate available carbohydrate ‘by difference’, the amount of dietary fibre is analysed and then subtracted from total carbohydrate (which itself can be determined by difference), i.e.:

$$\text{available carbohydrate} = \text{carbohydrate by difference} - \text{dietary fibre (FDA, 1993)}$$

This approach yields the estimated weight of available carbohydrate, but gives no indication of the composition of the various saccharides comprising available carbohydrate. It also includes the cumulative errors from the analytical measurements of other non-carbohydrate compounds. Available carbohydrate determined by direct analysis is the sum of analysed values for free sugars (glucose, fructose, galactose, sucrose, maltose, lactose and oligosaccharides) and complex carbohydrates (dextrins, starch and glycogen). In general, sugar analysis only measures the quantities of mono- and disaccharides. Oligosaccharides with a degree of polymerisation of two to nine can be quantitated by high-performance liquid chromatography (HPLC) techniques. However, available carbohydrate data currently used, (the sum of starch and total sugars), does not include oligosaccharides and/or maltodextrins. In most foods oligosaccharides are present in relatively low

quantities. In vegetables, however, and in some processed foods where glucose syrups and maltodextrins are added, oligosaccharides may make a significant contribution to carbohydrate content. In this situation, available carbohydrate determined by direct analysis may underestimate the carbohydrate content for those foods compared to the results derived from available carbohydrate 'by difference'. Overall, direct analysis, by summation of individual carbohydrates (Hicks, 1988; Southgate, 1991), is preferred to the assessment of available carbohydrate 'by difference' in the determination of energy content. New Zealand and Australia also use 'available carbohydrate' determined by direct analysis for the purposes of calculating and assigning energy factors.

Unavailable carbohydrate, on the other hand, is defined as the food carbohydrate components not digested by the endogenous secretions of the human digestive tract (McCance & Lawrence, 1929). The unavailable carbohydrate fraction includes the structural polysaccharides of the plant cell wall, pectins, cellulose, hemicelluloses, lignin (not a carbohydrate) and non-starch reserve polysaccharides such as inulin and the algal polysaccharides. Strictly speaking, lignin is not a carbohydrate but is wholly indigestible. In the UK, methods were developed in the 1930s for measuring unavailable carbohydrate by deducting the protein and starch contents (obtained after amyolytic hydrolysis using a fungal enzyme preparation) from the residue of the food insoluble in 80% v/v ethanol. The values obtained from these procedures were published in the 1st, 2nd and 3rd editions of *The Composition of Foods* (McCance & Widdowson, 1940; 1946; 1960). The method was developed further by Southgate during the period 1959-1969, using sequential hydrolysis and specific colorimetric methods to measure the major classes of components of plant cell walls (pentose, hexoses and uronic acids); lignin was determined by gravimetric analysis (Southgate 1969b). The values obtained from Southgate procedures were published in the 4th edition of *The Composition of Foods* (Paul & Southgate, 1978) and have been widely used since then. Though the Southgate method is simple relative to the amount of information it provides, it does not remove starch completely, and the colorimetric method for the measurement of individual sugars is non-specific. When gas chromatographic techniques became available for measuring the component sugars in early 1976, Englyst began to develop methods that were able to remove any interference from enzymatically resistant starch and to measure the non-alpha-glucans of the plant cell wall with precision. This approach includes a separate procedure for measuring enzymatically resistant starch formed during food processing, which would otherwise be included as non-starch

polysaccharide. This method is now referred to as the non-starch polysaccharide (NSP) method (Englyst *et al.*, 1982; Englyst & Cummings, 1988), and is used in the UK for food evaluation and food composition tables. The fraction measured by this procedure does not include lignin and resistant starch.

In the USA, the concept of dietary fibre was first introduced by Hipsley (1953) as a shorthand term for the constituents of the plant cell wall in the diet. It was later adopted by Trowell in 1972 designating the skeletal remains of the plant cell wall, which were rather non-specific. In 1976, the term dietary fibre was defined as ‘the sum of lignin and the plant polysaccharides that were not digested by the endogenous secretions of the human digestive tract (Trowell *et al.*, 1976). This definition was equivalent to ‘unavailable carbohydrate’ as defined by McCance and Lawrence, and enables one to use the methods developed for unavailable carbohydrates to quantify the dietary fibre in foods and diet. In the meantime more efforts were focused on the indigestibility of dietary fibre to develop so-called ‘physiological’ methods, which incorporated proteolytic and amylolytic enzymatic treatments to remove digestible components, leaving an indigestible dietary fibre residue. Asp and his co-workers developed the enzymatic procedures and proved that the approach could be used to provide a total dietary fibre value, to characterise the soluble and insoluble fractions, and to provide a residue suitable for more detailed analyses of the monosaccharide, uronic acid and lignin components of dietary fibre (Asp *et al.*, 1983). These procedures were developed further by an international group of authors into the ‘total dietary fibre’ (TDF) method of AOAC (Prosky *et al.*, 1984; 1988; AOAC, 2000). Overall, the TDF method of the AOAC (AOAC, 2000) and the NSP method of Englyst (Englyst & Cummings, 1988) have been generally accepted by AOAC International and the Bureau Communautaire de Reference (BCR) of the European Community (EC) as standard methods for dietary fibre determination. Both methods have undergone collaborative testing with satisfactory outcomes.

Table 1.11 shows the relationship between the different approaches to measuring and classifying dietary fibre in foods. In general, the gravimetric TDF values are slightly higher than the NSP values because the former include lignin and resistant starch. The differences are small for most vegetables and fruits, but may be of the order of 1g / 100g for unprocessed cereal foods due to the non-inclusion of lignin in NSP values. In heat-processed cereal foods and potato the TDF values may be 2-3g/100g higher because of the

inclusion of resistant starch. However, in most cases differences in precision and accuracy of both methods under routine conditions are not sufficient to state that the values are significantly different. As for TDF, Southgate values are generally higher than NSP values because they include substances measuring as lignin and also because the enzymatic preparation used, leaves some enzymatically resistant starch in the dietary fibre residue. A resistant starch value can be obtained from the NSP procedures but because this method uses different conditions and enzymes this may or may not be the same as the enzymatically resistant starch from the Southgate method. The Southgate values are similar in magnitude to the TDF values.

Table 1.11. Relationships between different ways of classifying and measuring the polysaccharides in foods (adapted from Johnson & Southgate, 1994).

Total dietary fibre (gravimetric)	Non-starch polysaccharides (NSP) Englyst fibre	Non-cellulosic Polysaccharides (NCP)	Other polysaccharides	Soluble fibre	Southgate fibre (unavailable carbohydrate)	Plant cell wall
			Pectin			
			Hemicellulose	Insoluble fibre		
	Cellulose	Cellulose				
	Lignin	Lignin				
	Enzymatically resistant starch					
Starch						

NB: Broken lines indicate boundaries that are not absolute.

As more has been learnt about plant fibre in foods, a variety of methods for analysis has been developed. Different methods measure different components of fibre, and thus give specific values. This situation affects not only the values in food composition tables for dietary fibre *per se*, but also those for available carbohydrate ‘by difference’. Overall, both available carbohydrate and unavailable carbohydrate are useful concepts in energy evaluation and carbohydrate should be analysed by a method that allows determination of both available and unavailable carbohydrate. For energy evaluation purposes, direct analysis is preferred to the method ‘by difference’ because the information on the carbohydrate components is useful in the determination of dietary energy content and for deriving energy factors. For food labelling purposes, ‘Dietary fibre’ is a useful concept that is familiar to consumers. The terms soluble and insoluble fibre are considered less useful in energy evaluation as the physical characteristic of solubility / insolubility does not strictly

correlate with fermentability / non-fermentability. Both the AOAC TDF method (AOAC 985.29) and the NSP method (Englyst & Cummings, 1988) can be used for dietary fibre analysis. Recent research data from Megazyme (Megazyme International Ireland Ltd.) show that the AOAC TDF method may underestimate the TDF content in some foods due to part of the resistant starch in the food becoming digestible after the heating step used in the assay. Therefore, the sum of non-starch polysaccharides (NSP) and resistant starch might be a better way to represent the dietary fibre content in food (BNF, 1990, Brown *et al.*, 1998).

1.5.2 Analytical methods for fats in foods

There is more agreement on standardized methods for analysis of dietary fat than there is for carbohydrate and protein. The fat content of foods is usually determined by one of three general methods: (1) simple extraction with a solvent, (2) acid or alkaline hydrolysis with extraction, and (3) analysis of fatty acids with expression as triglyceride equivalents. The first two methods are gravimetrically based; they measure the total fat content which includes phospholipids, wax esters, as well as minor amounts of non-fatty material (AOAC, 2000). Values obtained from these methods for 'total fat' are highly method-dependent, especially in the case of low-fat foods. Fat contents reported in most food tables such as the American Food table, UK Food table and New Zealand Food table are the weights of total fat, which include simple lipids (mono, di-, triglycerides and waxes), compound lipids (phospholipids, glycolipids and lipoprotein), and derived lipids (free fatty acids, sterols, carotenoids, and fat soluble vitamins). In the past total fat was determined by ether extraction, but modern methods lead to a more complete extraction. Thus, the heat of combustion of extracted lipid as currently determined might not be comparable to values reported by Atwater. Atwater and Bryant (1900) assigned 9.5 kcal/g (which is the heat of combustion value for a triglyceride) to the ether extraction fraction to compensate for incomplete extraction. However, most phospholipids have a heat of combustion nearer 7 kcal/g (Kinsella *et al.*, 1975). Preliminary data measured by Miles *et al* (1981) also showed that the heat of combustion for lipid extracted by chloroform-methanol (a more complete extraction of lipid than with ether) is in general lower than the values reported by Atwater for the fat fractions of similar products.

Analysing fat as fatty acids allows the assignment of more accurate heat of combustion values to lipids components. A more accurate estimate of the caloric contribution from the lipid fraction may be achieved. FAO (2003) recommended that total fat should be analysed as fatty acids and expressed as triglyceride equivalents, because this approach excludes waxes and the phosphate content of phospholipids, neither of which can be used for energy (James *et al.*, 1986). The gravimetric method, although less desirable, is still accepted for energy evaluation purposes (AOAC, 2000).

1.5.3 Analytical methods for the protein content in foods

For many years, the protein content of foods has been calculated on the basis of determined total nitrogen content, and the Kjeldahl or Dumas methods have been applied almost universally to determine nitrogen content (AOAC, 2000). Protein content is estimated by multiplying the nitrogen content with a factor, based on the nitrogen content of the predominating protein present in the foods. On the basis of early determinations the average nitrogen (N) content of proteins was considered to be about 16 percent, leading to the factor, 6.25. However, the nitrogen content of specific amino acids (as a percentage of weight) varies according to the molecular weight of that amino acid and the number of nitrogen atoms it contains. The nitrogen content of proteins actually varies from about 13 to 19 percent which could lead to the nitrogen conversion factors ranging from 5.26 (1/0.19) to 7.69 (1/0.13). In the course of extensive investigations, Jones (1931) prepared a set of special factors for converting nitrogen content to protein content in foods. Since then Jones' factors have been widely adopted. **Table 1.12** lists Jones' special N-protein factors. Not surprisingly, a factor directly based on amino acid composition would provide a more reliable conversion factor for individual foods than one based on total nitrogen content (Heidelbaugh *et al.*, 1975). However, when data on amino acid composition for each food are not available, protein contents reported in most food tables are still derived by applying the appropriate factor to the total nitrogen present.

The protein content obtained by applying an appropriate factor to the total nitrogen is influenced by other nitrogenous compounds, such as nitrates, nitrites, purine bases, choline, and free amino acids, which is referred to as the non-protein nitrogen (NPN). Only a small

part of NPN is available for the synthesis of amino acids. If the NPN fraction is not excluded from the protein fraction, the contribution of protein to the caloric value of food tends to be overestimated. Merrill & Watt (1973) noted, however, that the error in total energy estimation caused by such a calculation is very small because those foods (mostly fruits and vegetables) having a large proportion of nitrogen as NPN usually contain small amounts of total nitrogen. In the case of certain vegetables (e.g. potatoes) that contain a large proportion of non-protein nitrogen substances, this NPN is amino acid in nature and little error will be introduced in the use of a factor applied to the total nitrogen. For those foods that contain a significant amount of non-protein nitrogen in the form of urea, purines and pyrimidines (e.g. mushrooms), the non-protein nitrogen should be subtracted before multiplication by the appropriate factor (FAO, 2003). For mixed diets, the average factor of 6.25 used for calculating protein content from N content would only introduce errors less than 3%, as the average N content of diets tends to be close to 16%. Because protein contributes an average of about 15 percent of energy in most diets, the use of an average N-protein factor of 6.25 would not be expected to introduce an error of more than 1 percent in the estimation of the energy derived from protein (FAO, 2003).

Table 1.12. Specific (Jones) factors for the conversion of nitrogen content to protein content (adapted from Merrill & Watt, 1973).

Food	Factor	Food	Factor	Food	Factor
Animal origin:		Plant origin:		Plant origin:	
Eggs	6.25	Legumes:		Nuts:	
Gelatin	5.55	Beans:		Almonds'	5.18
Meat	6.25	Adzuki	6.25	Brazil	5.46
Milk	6.38	Castor	5.30	Butternuts	5.30
Plant origin:		Jack	6.25	Cashew	5.30
Grains & cereals:		Lima	6.25	Chestnuts	5.30
Barley	5.83	Mung	6.25	Coconuts	5.30
Corn (maize)	6.25	Navy	6.25	Hazelnuts	5.30
Millet	5.83	Soya bean	5.71	Hickory	5.30
Oats	5.83	Velvetbeans	6.25	Pecans	5.30
Rice	5.95	Peanuts	5.46	Pine nuts	5.30
Rye	5.83	Seeds:		Pistachio	5.30
Sorghum	6.25	Cantaloup	5.30	Walnuts	5.30
Wheat:		Cottonseed	5.30		
Whole kernel	5.83	Flaxseed	5.30		
Bran	6.31	Hempseed	5.30		
Embryo	5.80	Pumpkin	5.30		
Endosperm	5.70	Sesame	5.30		
		Sunflower	5.30		

NB: For groups of foods not included here, the conversion factor 6.25 should be used until more is known regarding their proteins.

Proteins are made up of chains of amino acids joined by peptide bonds, so they can be hydrolysed to their component amino acids, which can then be measured by ion-exchange, gas-liquid or high-performance liquid chromatography. Thus, the protein content of a food can be determined directly as the sum of the amino acids. This requires no assumptions to be made about NPN levels or the nitrogen content of amino acids. The value obtained from this approach represents a 'true protein' value for that food. FAO has recommended that the protein content of foods should be measured as the sum of individual amino acid residues (the molecular weight of each amino acid less the molecular weight of water) plus free amino acids, whenever possible (FAO, 2003).

1.5.4 Analytical methods for alcohol and other energy-yielding components

In addition to the three major energy-yielding components (protein, fat and carbohydrate), alcohol, polyols and organic acids can be a significant source of energy in some diets. The traditional method for alcohol determination is the distillation-specific gravity method (D-SG) (AOAC, 1975), but a GLC based method (Foo *et al.*, 1978) or enzymatic procedures employing alcohol dehydrogenase (Bergmeyer, 1974) are preferable and more specific.

Organic acids are minor constituents of many foods, but in some the concentrations of individual acids may be nutritionally significant. Acetic acid is present in vinegar and consequently all pickled foods. It is also found in fermented food products. Citric and malic acids are the major organic acids in most fruits, vegetables and their products. Many manufactured products contain added citric acid. Lactic acid is present in fermented foods such as yogurt, sauerkraut and cheeses. Oxalic acid, often as the calcium salt, is found in many vegetables as well as in some fruits and nuts. It has no nutritional value. Tartaric acid is the major organic acid in wines and in the fruit of the tamarind. Of the available methods for analysis of organic acids, assays with specific enzyme techniques (Bergmeyer, 1974) appear to be preferable, although ion-exchange chromatography or TLC followed by a colorimetric procedure also gives satisfactory results (Stahl, 1965). With modern technology, high performance liquid chromatography (HPLC) becomes the preferred method for the determination of organic acids (Wills *et al.*, 1983).

'Polyols' is the term used to identify the monomeric, dimeric, and oligomeric sugar alcohols. Polyols are often used as food ingredients to replace sucrose for sweetness or are used for technical reasons. Polyols can be quantitated by high performance liquid chromatography (HPLC) techniques (Wills *et al.*, 1987). Sorbitol in food can also be measured by gas chromatography (AOAC, 1984).

1.6 Overall conclusion and inferences from the review of literature

As discussed in the previous sections, accurately predicting the energy content of foods or diets available for humans by calculation depends on the following factors:

1. The quantities of specific food or dietary components that provide energy should be determined by appropriate analytical methods;
2. The quantity of each individual component must be able to be converted into energy content using either a set of generally accepted factors or an appropriate empirical formula that corresponds to the energy-producing potential of the components in the human body;
3. The energy content of all components must be added together in such a way that the sum represents the amount of food energy physiologically available to the body.

In theory, the energy yield can be calculated 'exactly', given a complete chemical description of a food and its final metabolic products; however, such information is rarely available for individual foods and mixed diets. In practice, values for metabolizable energy can be assigned to foods or mixed diets by various systems that employ certain assumptions concerning the composition of foods, their metabolic products as well as digestion, absorption and the amount of energy available through metabolic conversions (Allison & Senti, 1983). The work carried out by Atwater and his colleagues at the turn of the twentieth century (Atwater & Bryant, 1900) has formed the foundation for predicting the metabolizable energy of foods based on their energy-containing components. The system is referred to as the Atwater system. Since then several different approaches have been taken in developing different systems to accommodate advances in knowledge since Atwater's time. Section 1.4 has listed most of the food energy systems derived to date. It has been recognized for some time that it might be physiologically inaccurate to assume that all

energy-yielding nutrients and dietary combinations can be treated as equivalent by correcting their heats of combustion for faecal and urinary losses as is done in the Atwater system. When a large amount of dietary fibre is included in the diet, the ME of the diet may not simply be additive from the energy content of the individual components, but is rather a function of the interaction of these nutrients with dietary fibre.

Concerns, particularly over the energy availability from dietary fibre has led to studies on evaluation of the accuracy of current food energy assessment systems (Southgate & Durnin, 1970; Allison & Senti, 1983; Livesey 1989; 1990; 1991a; Brown *et al.*, 1998; FAO, 2003). Many studies have also been conducted with humans and animals to compare the effect of different types and levels of dietary fibre on the digestibility of energy and nutrients, protein utilization, and colonic fermentation (Baer *et al.*, 1997; Cummings, 1980; 1983; Ehle *et al.*, 1982; Hillman *et al.*, 1983; Miles *et al.*, 1988; Miles, 1992; Tetens *et al.*, 1996; Wisker *et al.*, 1992; 1996a; 1997; 2000). Results from extensive analysis of the literature have shown (Livesey, 1990) that both Atwater general factors (1910) and McCance & Widdowson (1946) factors (also called British factors) do not adequately predict availability of dietary energy for diets rich in unavailable carbohydrate (Livesey, 1990; BNF, 1990; Wisker & Feldheim, 1993). This is of considerable importance, since these two systems form the basis of food energy assessment used in almost all regions of the world. Many systems have inherent limitations in the relevance of the derived energy values. Results from balance experiments demonstrate: 1) that the digestibility of energy-containing nutrients is significantly lower when a diet contains high levels of dietary fibre; 2) that changing from a diet of low dietary fibre to one of high dietary fibre changes microbial growth in the bowel and source of dietary fibre has different effects on intestinal flora; 3) that the breakdown of dietary fibre via fermentation in the colon may potentially contribute metabolizable energy to the body by supplying SCFA to the body, but the quantity of energy produced may vary with fibre composition. Based on published results it is reasonable to conclude: 1) that for mixed diets a digestible energy value of 8.4 kJ (2 kcal) is an appropriate value for unavailable carbohydrate; 2) that among published models the FDA factor (1993), Livesey's factor and Livesey's formula (1990) give little bias in predicting the metabolizable energy value of diets, especially for high fibre diets; 3) that the most precise and least biased approach for calculating the ME value of diets might be the empirical model, which is based on the dietary measurement of gross energy. Unfortunately an established database for the gross energy contents of all foods is still not available. In spite

of the above tentative conclusions regarding empirical and factorial approaches to predicting dietary ME, there is a limited amount of information concerning their accuracy in relation to very high fibre diets. Moreover, high fibre low fat diets and specialised foods are becoming more commonplace with an increasing emphasis on body weight-loss.

Thus the objective of the study reported in this dissertation was to evaluate the accuracy of empirical and factorial predictions of dietary ME for high fibre, low fat mixed diets. Three diets were formulated varying in fat and fibre content, and fibre origin. The systems chosen included four factorial models (Atwater general factors (1910), British approach (Holland *et al.*, 1991), FDA factors (1993), and Livesey factors (1990)) and five empirical models (Levy *et al.* (1958), Miller & Payne (1959), Miller & Judd (1984), Southgate (1975) and Livesey (1990)). The assessment was conducted by comparing determined ME values with those predicted by the above mentioned systems.

A second aim of the current study was to assess differences in nutrient digestibility, and urinary nitrogen and energy excretion among the different diets. When Atwater set up the Atwater system for estimating the ME value of foods, the assumption was made that each component of a food could be assigned an energy factor that is fixed and does not vary according to the proportions of other components in the food or diet. Atwater also assigned an average value of 7.9 kcal per gram of urinary nitrogen, which corresponds to 1.25 kcal per gram of protein absorbed, for the energy losses in the urine due to incomplete oxidation of protein in the body. As dietary fibre increases and more low-calorie foods are used, these assumptions are questionable. According to data from the Royal College of Physicians (1983), a 1% change in dietary energy intake would be associated with a nearly 5% change in the incidence of diabetes, coronary heart disease deaths and overall premature mortality. As little as 0.2% of food energy intake above requirements over the first four decades of life can result in obesity (Norgan, 1990; Webster, 1992; MaCracken, 1992). Therefore a fine control on energy intake plays a key role in the control of body weight and long-term health.

Chapter 2 Variation in the apparent faecal digestibility of macronutrients and urinary energy excretion for three diets varying in fat and fibre content – Assessment of the Atwater factors and related energy conversion factors *

2.1 Introduction

The incidence of obesity and related health disorders in developed nations is a matter of major concern (FAO/WHO/UNU 2004). One of several approaches to combating the development of obesity is the inclusion of specialised low-energy foods in the diet, and careful monitoring of individuals' dietary energy intakes versus energy expenditures. To develop such foods and dietary regimens, however, implies the need to be able to determine the 'available' energy contents of foods with accuracy, such that foods or components of a food with different bioavailable energy contents are able to be differentiated.

Commonly, the Atwater general factors (Merrill & Watt, 1973), though not originally intended to be used so generically, are applied to estimate metabolizable energy (ME) contents of foods (CAC, 1993). They are allowed to be used in the USA for food labelling purposes (USDA, 1998). Such a system assumes that the ME of a diet can be accurately predicted based on a few chemical components (crude protein, fat, carbohydrate) and that the ME of each component is constant across foods and food ingredients. Implicit in this assumption is that the ME content of a food is affected by the fat, carbohydrate and protein contents only; that the ratio of gross energy (GE) to either fat, protein or carbohydrate is constant across foods; that differences among foods and food ingredients in the degree of digestibility of each of fat, carbohydrate and protein are quantitatively unimportant; and that differences in the urinary excretion of energy per unit dietary protein are quantitatively unimportant.

The present study, in which ME was determined in subjects receiving a refined diet (high fat, low fibre) and two high fibre diets (cereal or fruit/vegetable based), allowed an assessment to be made of differences in nutrient digestibility and urinary energy excretion among diverse types of diet. Comparison was made between the prediction of ME based on

* This work has been accepted for publication in the American Journal of Clinical Nutrition.

the Atwater system and determined ME. The predictive accuracy of several other previously published factorial or empirical models for predicting food ME was also assessed.

2.2 Subjects and methods

2.2.1 Subjects

Twenty-seven adult subjects (**Table 2.1**) completed the study. Subjects were visibly healthy, had no gastrointestinal problems and were not receiving any medication. None of the subjects had received antibiotics for at least three months prior to the study. Subjects were asked to record their normal food intake for a period of four days prior to the study, to allow calculation of daily food energy intake (**Appendix 6**). The 24-hour recall method was also used to assess each subject’s habitual daily food intake and normal daily activity level (**Appendix 6**).

Written informed consent (**Appendix 5**) was obtained from all participants and confidentiality was maintained throughout the study. Approval to conduct the study was obtained from the Massey University Human Ethics Committee (HEC 98/123) (**Appendix 8**). Subjects were well educated and highly motivated.

Table 2.1. Physical characteristics for each group of subjects receiving one of three experimental diets¹.

		Refined Diet	Fruit & Vegetable Diet	Cereal Diet
No. of subjects	Total	9	9	9
	Female	5	5	5
	Male	4	4	4
Age (years)	Mean	35.9	35.2	38.8
	SE ²	2.1	3.7	3.9
	Range	26-46	24-59	23-52
Height (cm)	Mean	168.8	168.1	169.6
	SE	3.3	2.5	3.5
	Range	155.2-188.1	155.4-179	154-180
Body Weight (kg)	Mean	73.4	75.8	71.2
	SE	6.5	7.2	4.9
	Range	54.3-120.4	52.2-123	45.8-91.6

¹There were no significant differences in physical characteristics among groups.

²SE: standard error.

2.2.2 Diets

Three test diets (**Appendix 2**) were formulated from food ingredients that have relatively constant chemical compositions and that are easy to handle, store and cook. Diet one, designated 'the refined diet' contained mainly high fat low fibre foods (viz. white bread, butter, peanuts, homogenized milk, shaved ham, cookies, chocolate bars, cheese & mince pie). Diet two, designated the 'fruit and vegetable diet' contained high amounts of dried fruit and vegetables (viz. dried apricots, sultanas, prunes; fruit juice, fruit bread), while diet three, designated the 'cereal diet' contained high amounts of cereal based foods (viz. wholemeal bread, bran cereal, crisp bread, bran & apple muffin). The twenty-seven volunteers were randomly allocated to the three test diets (9 subjects per diet). Each subject selected a dietary intake level designed to approximately meet his or her daily energy requirement. During this parallel study subjects were free living at home and consumed a fixed daily intake of provided food.

Although dietary intake was individualised for each subject, the proportions of the nutrients within any one diet and thus the proportion of energy derived from the respective energy-yielding nutrients remained the same within dietary treatments regardless of differences in absolute intake levels. Subjects were clearly instructed (**Appendix 7**), and this was reinforced throughout the study, that no extra food, alcoholic or other energy-containing beverages were permitted during the experimental period. Tea, coffee, pepper, salt and sugar were supplied to each subject, with each subject accurately recording the actual quantities of these items consumed daily. At the end of the study, each subject's actual dietary energy intake was corrected by taking account of the amount of tea, coffee and sugar ingested and any food not consumed.

2.3 Experimental procedure

The metabolic study lasted ten days and included a four-day adjustment period followed by a six-day quantitative balance period whereby faeces and urine were collected. Urine samples were acidified at collection (6M HCl). Faecal and urine samples were kept on ice after collection and were transferred to the laboratory twice daily, where individual daily

samples were weighed and then stored frozen (-20°C). Subsequently, faeces were thawed, bulked over days, freeze dried, finely ground (1mm mesh) and mixed. Urine was also bulked over days for each subject.

Representative samples of freeze-dried faeces and urine for each subject were taken for chemical analysis.

On five occasions during the experimental period an entire day's duplicate meals (as eaten) were collected for each diet to allow determination of the dietary nutrient composition. The food was homogenised, weighed and frozen (-20°C). Composite samples of each individual dietary ingredient were freeze-dried and finely ground (1mm mesh).

2.4 Chemical analysis

All freeze-dried samples including diets, faeces, uneaten food and food ingredients were analysed in duplicate for dry matter (DM), ash, total nitrogen (N), total fat and gross energy (GE). Urine samples were analysed for GE and nitrogenous constituents. The overall mean coefficients of variation (CV%, between duplicates within samples) were 0.1, 1.1, 0.9, 0.9 and 4.9% for DM, ash, N, GE and total fat, respectively. External standards were included for each assay and recoveries ranged from 95 to 105%.

The DM and ash contents of the faeces and food samples were determined after drying the samples in an oven at 105°C for 16 hours, followed by ashing in a Muffle furnace at 500°C for 16 hours (AOAC, 2000). The DM contents of the total diet samples, which contained large amounts of sugar and fat, were determined by drying in a 70°C vacuum oven until a constant weight was achieved (approximately 24 hrs) (AOAC, 2000).

Total nitrogen (N) was determined on both food and faeces samples by using the Dumas methods on a LECO FP3000 CNS auto analyser (Bellomonte *et al.*, 1987; AOAC, 2000). The N content of urine samples was determined on a Kjelteltech 1030 auto analyser (Tecator, Sweden) by following a standard Kjeldahl procedure (AOAC, 2000). Crude protein was calculated as total N x 6.25, with the exception of coffee for which the conversion factor

5.3 was used (USDA, SR13). Amino acids were measured by using ion-exchange HPLC after hydrolysis of the samples in 6M HCl for 24 h at 110 °C in evacuated sealed tubes. Methionine and cysteine were measured as methionine sulfone and cysteic acid, respectively, after hydrolysis of samples that had been oxidized by using performic acid. Tryptophan was not measured. Ammonia, urea, uric acid and creatinine in each urine sample were determined on a Cobas Fara II autoanalyser (Hoffman-La Roche, Basel, Switzerland), following the procedures outlined by Tiffany *et al* (1972), Fossati *et al* (1980) and Larsen (1972), respectively. The Soxhlet method (AOAC, 2000) was used to measure the fat content of dietary faecal samples. All faecal samples were dried overnight in a 60°C oven, followed by extraction with petroleum spirits (40°C-60°C) for 7 hours (AOAC, 2000). For the dietary samples the material was acid hydrolysed (3M HCl) before the fat extraction (AOAC, 2000). The fat content of tomato sauce was determined following the Mojonnier method (AOAC, 2000).

A one-step extraction-transesterification procedure was used for determining the total fatty acids in the dietary and faecal samples (Sukhija *et al.*, 1988). Samples (50-300 mg) of freeze-dried material containing 10-50 mg of fatty acids were treated with a solvent mixture consisting of methanol-toluene-acetyl chloride (27:20:3) at 70°C for 2h. The organic layer was transferred into a screw-cap culture tube (Kimax, Kimble Glass Inc., New Jersey, USA), dried and the pigments removed by adding anhydrous sodium sulphate and florisil. The fatty acid composition was then determined by gas chromatography (Shimadzu GC-8A, packed column with 15% Eggs-X on chromosorb W, 100-120 mesh) with nitrogen as the carrier gas, FID detector and using pentadecanoic acid (C15:0) as an internal standard.

Gross energy contents of the samples were determined using an adiabatic bomb calorimeter (Gallenkamp Co. Ltd, London, Great Britain) (Miller & Payne, 1959).

The amount of total carbohydrate (%) in the diets and faeces was defined as the difference between 100 and the sum of the percentage of water, protein, total fat and ash (Henneberg *et al.*, 1860; 1864).

The amounts of total, soluble, and insoluble dietary fibre in the dietary samples were analysed using an enzymatic-gravimetric method (AOAC, 2000). Duplicate samples of freeze-dried diets (fat extracted when containing >10% fat) underwent sequential enzymatic

digestion by heat stable α -amylase, protease, and amyloglucosidase to remove starch and protein. For total dietary fibre (TDF), the digested material was treated with four volumes of ethanol to precipitate soluble dietary fibre before filtering, and the TDF residue was washed consecutively with 78% ethanol, 95% ethanol and acetone, dried and weighed. For insoluble dietary fibre (IDF), the digested material was filtered, and the residue (IDF) was washed with warm water, dried and weighed. The filtrate and washes from the IDF procedure were then combined and treated with 95% ethanol to allow soluble dietary fibre (SDF) to precipitate. SDF residue was then filtered, dried and weighed. On all occasions one of the dried residues was analysed for protein, while the other was incinerated at 525°C to determine the ash content. TDF, IDF, and SDF are the respective weights of the residues from the TDF, IDF and SDF procedures less the weights of protein, ash, and material from the blank for the TDF, IDF and SDF procedures, respectively.

Total non-starch polysaccharide (NSP) in the dietary samples was determined as described by Englyst and co-workers (Englyst & Cummings, 1984; 1988). Starch was gently removed by incubating with thermostable α -amylase, and β -glucanase-free amyloglucosidase. The polysaccharides in the starch-free residues were dispersed in 12M H₂SO₄ at 35°C for 30 min; followed by hydrolysing with 2M H₂SO₄ in a boiling water bath for 1 hour. The constituent sugars released by acid hydrolysis procedures were then measured by gas chromatography (GC), to give values for individual monosaccharides. The hydrolysate was also analysed for uronic acids. The hydrolysate was mixed with a sodium chloride / boric acid solution and concentrated sulphuric acid, incubated at 70°C for 40 min, then dimethylphenol was added prior to absorbance being measured on a spectrophotometer. Total NSP was equal to the sum of neutral sugars and uronic acids expressed as polysaccharide.

The level of available carbohydrate in the dietary samples was determined as the sum of the individual mono- and disaccharides and starch expressed as the weight of the carbohydrate (Athar *et al.*, 2003). Sugars were extracted with aqueous alcohol followed by derivatisation with Tri-Sil Z (TMS-Imidazole in pyridine, Pierce Ltd, Germany), then measured by gas chromatography (Simadzu GC, OV17 column, temperature program 170°C-240°C at 5°C/min, N₂ as carrier gas). Starch was determined using a commercial kit (Total Starch Kit AA/AMG, Megazyme Australia, Sydney, Australia) following a standard procedure (AOAC, 2000). Samples were completely dissolved in dimethylsulfoxide, and hydrolysed

with thermostable α -amylase, and amyloglucosidase. The amount of released glucose was determined by spectrophotometry using Chromogen reagent (GOPOD) purchased from Megazyme international Ireland Ltd. The level of available carbohydrate was also calculated as the sum of the individual mono- and disaccharides and starch but expressed as monosaccharide equivalents (Holland *et al.*, 1991).

Resistant starch (RS) in the dietary samples was measured using a commercial kit (Resistant Starch Kit, Megazyme Australia, Sydney, Australia) following a standard procedure (AOAC 2002.02). Samples (defatted first if fat content > 10%) were incubated at 37°C in a shaking water bath with pancreatic α -amylase and amyloglucosidase (AMG) for 16 hours to remove non-resistant starch. The RS was then recovered as a pellet upon centrifugation and was dissolved in 2M KOH. This solution was then neutralized and hydrolysed with AMG. The RS content was quantified by measuring the amount of released glucose with glucose oxidase/peroxidase reagent (GOPOD) (AOAC, 2005).

2.5 Data analysis

Dietary ME values were calculated using the following equation (Merrill & Watt 1973):

$$\text{ME(MJ/kg)} = \frac{\text{GE food (MJ/d)} - \text{GE faeces (MJ/d)} - \text{GE urine (MJ/d)}}{\text{Food Intake (kg/d)}}$$

Apparent faecal energy digestibility was calculated as follows:

$$\text{Apparent digestibility of energy (\%)} = \frac{\text{GE food (MJ/d)} - \text{GE faeces (MJ/d)}}{\text{GE food (MJ/d)}} \times \frac{100}{1}$$

Analogously, the apparent digestibilities of dietary fat, N and carbohydrate were calculated as:

$$\text{Apparent digestibility of nutrient (\%)} = \frac{\text{Nutrient food (g/d)} - \text{Nutrient faeces (g/d)}}{\text{Nutrient food (g/d)}} \times \frac{100}{1}$$

The mean digestibilities for gross energy, crude protein, fat, the fatty acids and total carbohydrate were compared among the three diets following the PROC GLM procedure for a one-way analysis of variance (ANOVA) using the statistical program SAS (version 9,

SAS Inst., Inc., Cary, NC). When significant differences were found after applying ANOVA, the respective treatment means were compared using the Tukey test. Results were considered statistically significant at $P < 0.05$.

Student's t test was used to compare determined with calculated ME values for each of the three diets, respectively. Differences were considered statistically significant at $p < 0.05$.

2.6 Results

The determined gross chemical compositions of the three experimental diets are given in **Table 2.2** and the determined compositions of the non-starch polysaccharide (NSP) fractions, the amino acid and fatty acid compositions for each diet in **Tables 2.3, 2.4 & 2.5**.

Table 2.2. Determined chemical composition of the three experimental diets (g/100g dry matter).

Component	Refined Diet	Fruit & Vegetable Diet	Cereal Diet
Moisture	58.31	55.66	65.96
Crude protein	17.62	13.13	15.83
Fat	27.59	13.13	10.91
Ash	3.21	3.26	4.18
Total CHO ¹ by difference ²	51.57	70.48	69.08
Ava CHO by difference ³	46.66	62.59	59.06
Sugars	20.14	34.74	26.56
Starch	25.72	23.47	28.59
Ava.CHO as carbohydrate weight	45.87	58.21	55.15
Ava CHO as monosaccharide equivalents	49.36	60.93	58.78
TDF ⁴	4.90	7.89	10.02
IDF ⁴	4.54	6.83	9.94
SDF ⁴	0.36	1.06	0.07
NSP ⁵	3.51	5.64	7.82
Resistant Starch	0.45	0.81	0.64

¹CHO represents Carbohydrate.

²Total CHO by difference = $100 - H_2O\% - Fat\% - CP\% - Ash\%$

³Ava. CHO by difference = Total CHO by difference - TDF%.

⁴TDF: total dietary fibre, IDF: insoluble dietary fibre, SDF: soluble dietary fibre, as determined by the AOAC method (AOAC, 2000).

⁵NSP represents the total non-starch polysaccharides, determined by the method of Englyst and Cummings (1988).

As expected, the fruit and vegetable and the cereal diet were lower in crude protein and fat compared to the refined diet but had considerably higher fibre levels. The fruit and vegetable diet contained the highest level of uronic acid (20.7% of NSP) compared with 14.2% and 8.7% for the refined and cereal diets, respectively. High amounts of arabinose (27.5% of NSP), xylose (35.8% of NSP) and glucose (19.2% of NSP) in the cereal diet indicate that the cereal fibre mainly comprised cellulose and hemicellulose.

Table 2.3. Chemical constituents of the non-starch polysaccharides (NSP)¹ expressed as percentage of total non-starch polysaccharides.

Component	Refined Diet	Fruit & Vegetable Diet	Cereal Diet
Arabinose	23.1	19.7	27.5
Xylose	23.6	16.1	35.8
Mannose	5.1	11.5	2.0
Galactose	10.3	15.4	6.8
Glucose	23.6	16.3	19.2
Uronic acid	14.2	20.7	8.7

¹As determined by the Englyst method.

Table 2.4. Amino acid profiles for the three experimental diets (g/100g dry matter)¹.

Amino acid	Refined Diet	Fruit & Vegetable Diet	Cereal Diet
Aspartic acid	1.42	1.27	1.13
Threonine	0.51	0.42	0.47
Serine	0.66	0.48	0.60
Glutamic acid	3.85	2.66	3.41
Proline	1.26	1.05	1.17
Glycine	0.64	0.43	0.51
Alanine	0.68	0.53	0.64
Valine	0.76	0.58	0.67
Isoleucine	0.63	0.48	0.55
Leucine	1.22	0.91	1.02
Tyrosine	0.64	0.44	0.50
Phenylalanine	0.77	0.56	0.64
Histidine	0.47	0.39	0.50
Lysine	0.77	0.49	0.71
Arginine	1.02	0.67	0.72
Cysteine	0.24	0.18	0.25
Methionine	0.29	0.24	0.29

¹Amino acids were determined by acid hydrolysis followed by HPLC separation (AOAC, 2000).

Table 2.5. Fatty acid compositions for the three experimental diets (g/100g dry matter)¹.

Fatty acid	Refined Diet	Fruit & Vegetable Diet	Cereal Diet
<u>SFA</u>			
8:0	0.165	0.457	0.051
10:0	0.329	0.353	0.101
11:0	0.036	0.006	0.010
12:0	0.455	2.287	0.137
13:0	0.018	0.004	0.005
14:0	1.339	1.088	0.431
16:0	5.463	1.754	2.143
17:0	0.211	0.093	0.082
18:0	2.603	1.055	0.860
20:0	0.138	0.022	0.026
21:0	0.098	0.029	0.033
22:0	0.219	0.009	0.023
23:0	0.012	0.004	0.014
24:0	0.122	0.007	0.012
<u>MUFA</u>			
14:1n-5c	0.116	0.027	0.038
15:1n-5c	0.001	ND	ND
16:1n-7c	0.277	0.113	0.115
17:1n-7c	0.001	ND	0.001
18:1n-9t	0.144	0.019	0.020
18:1n-7t	0.334	0.109	0.094
18:1n-9c	6.832	2.129	1.931
18:1n-7c	0.145	0.071	0.081
20:1n-9c	0.123	0.023	0.029
22:1n-9c	0.009	0.003	0.003
24:1n-9c	0.002	0.001	0.002
<u>PUFA</u>			
18:2n-6t	0.009	ND	ND
18:2n-6c	2.794	0.811	2.223
18:3n-6c	0.012	0.010	0.029
18:3n-3c	0.231	0.214	0.388
20:2n-6c	0.009	0.003	0.006
20:3n-6c	0.006	0.002	0.003
20:3n-3c	0.003	0.001	0.001
20:4n-6c	0.009	0.004	0.004
20:5n-3c	0.017	0.004	0.008
22:2n-6c	0.015	0.003	0.005
22:6n-3c	0.005	ND	0.007
<u>Fatty acid ratios (% of total fatty acid)</u>			
SFA	50.3	66.9	44.0
MUFA	35.8	23.3	26.0
PUFA	14.0	9.8	30.0

¹ SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. Fatty acids were determined using gas chromatography.

The mean values for daily energy intake and excretion are given in **Table 2.6**. Energy intake varied considerably within each group, with men tending to have higher energy intakes than women, and people with higher body weight tending to eat more. However, energy intake was not always strictly proportional to body weight. Faecal and urinary energy excretions were also highly variable among subjects on a diet. Data for faecal bulking (mg wet or dry faecal excretion/g food dry matter intake) are also given in Table 2.6, showing that faecal bulking (based on dry faecal weight) was significantly ($P<0.05$) higher for the cereal diet in comparison with either the refined or fruit and vegetable diet.

Table 2.6. Mean (n=9) daily energy intakes and excretions for the subjects receiving the three experimental diets¹.

		Refined Diet	Fruit & Vegetable Diet	Cereal Diet
Daily intake (MJ/day)	Mean	12.250 ^a	10.961 ^a	9.753 ^a
	SE	0.94	0.93	1.06
	Range	9.056-16.790	6.570-13.200	7.243-14.625
Faecal excretion (MJ/day)	Mean	0.801 ^a	1.119 ^a	1.067 ^a
	SE	0.06	0.16	0.12
	Range	0.549-1.086	0.529-1.903	0.723-1.637
Urinary excretion (MJ/day)	Mean	0.388 ^a	0.395 ^a	0.417 ^a
	SE	0.02	0.03	0.04
	Range	0.281-0.468	0.287-0.497	0.268-0.651
Faecal bulking (mg wet Faecal weight /g DM ² intake)	Mean	257.6 ^a	440.4 ^{ab}	559.8 ^b
	SE	25.9	82.6	32.0
	Range	163.3 – 365.3	215.7 – 916.4	443.9 – 753.1
Faecal bulking (mg dry Faecal weight /g DM intake)	Mean	66.2 ^a	82.3 ^a	108.9 ^b
	SE	2.3	7.3	2.3
	Range	54.5 – 78.0	53.7 – 125.4	100.8 – 121.8

¹a,b,c Means with different superscripts within the same row were significantly ($P<0.05$) different.

²DM: Dry matter.

The daily food N intakes and excretions of N are given in **Table 2.7**, along with the nitrogenous composition of the urine and the urinary energy excretions expressed relative to N intake and excretion. The urinary energy per unit urinary N was significantly ($P<0.05$) higher for the fruit and vegetable diet (44.1 kJ/gN) compared to the cereal diet (37.6 kJ/gN) which was significantly higher ($P<0.05$) than that for the refined diet (33.9 kJ/gN). Urea was the major urinary N constituent. Urinary creatinine nitrogen excretion was higher ($P<0.05$) for the fruit and vegetable diet compared to the other two diets, while the uric acid nitrogen content was significantly higher ($P<0.05$) for the cereal diet in comparison with the refined and fruit and vegetable diets.

Mean values for the apparent faecal digestibility of gross energy, crude protein, fat, total carbohydrate (by difference) and the individual fatty acids are given in **Table 2.8**. The digestibility of energy was statistically significantly lower for subjects receiving the higher fibre fruit and vegetable and cereal diets compared to the refined diets (maximum difference = 4.4% units). The digestibility of crude protein was also significantly lower ($P < 0.05$, maximum difference = 8.6% units) for the high-fibre diets as was the digestibility of fat (maximal difference = 8.7% units) for the fruit and vegetable diet and the digestibility of total carbohydrate which was significantly ($P < 0.05$) lower for subjects receiving the cereal diet (maximum difference = 4.4% units). There were quantitatively important and statistically significant ($P < 0.05$) differences in the apparent faecal digestibilities of the individual dietary fatty acids. Of particular note were the very low digestibilities for the C12:0, C14:0 and C18:0 fatty acids in the fruit and vegetable diet. Palmitoleic acid (C16:1) was digested completely for all three diets, however the medium chain fatty acids (C10:0 to C16:0) were digested almost completely only for the refined and cereal diets. The observed digestibility of the C18:0 fatty acid appeared to be lower for all of the diets than the digestibility of the remainder of the fatty acids, though its unsaturated isomers (C:18.1, C:18.2, C:18.3) were well digested for each of the three diets.

The estimates of energy and nutrient digestibility were considerably more variable for the fruit and vegetable diet compared to the other two experimental diets. For some individuals on this diet as little as 75 percent of some fatty acids disappeared from the alimentary canal during digestion. It may indicate that the dietary fibre in the fruit and vegetable diet has effects on the fermentation process in the large intestine.

ME values for each diet were either determined experimentally or predicted based on chemical composition using different factorial models or empirically-based predictive equations (refer **Table 2.9**), and the determined and predicted values are given in **Table 2.10**. The differences between the determined and predicted ME values expressed as a percentage of determined ME for the diets are also included in **Table 2.10**.

The mean determined ME value for the refined diet was significantly higher ($P < 0.001$) than for the other two diets, which accords with its much higher fat and low fibre content. The mean determined ME's for the fruit and vegetable and cereal diets, however, were similar regardless of the difference in their fibre levels and origin. Determined ME was 0.90 of

determined GE for the refined diet, 0.86 determined GE for the fruit and vegetable diet and 0.85 determined GE for the cereal diet, demonstrating the significant contribution the gross energy value makes to ME.

It is clear from the results presented in Table 2.10 that application of the Atwater factors led to statistically significant and practically relevant differences between predicted and determined ME values for the three diets tested. The use of FDA and British modified Atwater factors, where dietary carbohydrate is corrected for insoluble dietary fibre (FDA) or is determined as available carbohydrate (British), gave better agreement but practically important differences (4%) remained. In general, the empirically derived prediction equations that were tested did not lead to a higher level of accuracy of prediction compared to the factorial models.

Table 2.7. Mean (n=9) daily food total nitrogen (N) intakes and N excretions, the composition of urinary nitrogen, the ratio of urine energy to urine N, and the ratio of urine energy to food N for subjects receiving the three experimental diets.

	Refined Diet			Fruit & Vegetable Diet			Cereal Diet		
Intake and excretion of N (g/d)	Mean	SE	Range	Mean	SE	Range	Mean	SE	Range
Daily Intake	14.74 ^a	1.15	10.85-20.22	11.63 ^a	1.00	6.97-13.99	12.45 ^a	1.35	9.29-18.67
Faecal Excretion	1.45 ^b	0.31	1.12-1.87	2.18 ^a	0.23	1.11-3.03	2.13 ^a	0.22	1.41-3.41
Urinary Excretion	11.48 ^a	2.06	8.26-14.33	8.94 ^b	0.55	6.43-11.25	11.11 ^{ab}	1.14	6.96-17.63
Urinary nitrogenous constituent (g/100g total urinary N)									
Creatinine N	3.5 ^a	0.1	3.0-4.2	4.4 ^b	0.2	3.8-5.5	3.7 ^a	0.2	2.9-4.4
Ammonia N	3.8 ^a	0.1	3.2-4.3	3.9 ^a	0.3	2.9-5.2	3.9 ^a	0.3	2.9-5.4
Uric acid N	0.8 ^a	0.1	0.5-1.5	0.8 ^a	0.2	0.5-1.2	1.3 ^b	0.2	0.9-1.6
Urea N	87.4 ^a	0.3	86.0-89.4	85.4 ^a	2.0	80.3-96.1	85.0 ^a	0.3	82.2-89.9
Urine energy : Urine N (KJ/gN)	33.9 ^a	0.3	32.5-35.4	44.1 ^b	0.9	39.1-47.7	37.6 ^c	0.4	35.2-39.1
Urine energy : Food N (KJ/gN)	26.9 ^a	1.2	19.8-30.8	34.9 ^b	1.8	23.8-42.9	33.9 ^b	1.2	28.3-39.6

^{a,b,c} Means with different superscripts within the same row were significantly ($P < 0.05$) different.
SE: Standard error.

Table 2.8. Mean (n=9) apparent faecal digestibility of gross energy, crude protein, fat, total carbohydrate and individual fatty acids for the three experimental diets.

	Refined Diet			Fruit & Vegetable Diet			Cereal Diet		
	Mean	SE *	Range	Mean	SE *	Range	Mean	SE *	Range
Digestibility of energy %	93.5 ^a	0.2	92.4-94.1	90.0 ^b	0.8	85.4-93.4	89.1 ^b	0.2	87.9-90.0
Digestibility of protein %	90.0 ^a	0.4	88.4-92.6	81.4 ^b	0.9	78.7-85.6	82.7 ^b	0.7	80.3-86.7
Digestibility of fat %	95.7 ^a	0.3	94.3-97.3	87.0 ^b	1.0	81.7-91.6	95.1 ^a	0.3	93.4-96.9
Digestibility of total CHO %	94.4 ^a	0.4	92.9-96.1	95.5 ^a	0.7	91.1-97.4	91.1 ^b	0.3	89.9-92.1
Digestibility of individual Fatty acids %									
¹ SFA: 10:0	99.9 ^a	0.1	99.3-100.0	87.4 ^b	1.5	81.6-93.0	98.2 ^a	0.9	94.1-100.0
12:0	98.7 ^a	0.8	93.1-100.0	80.7 ^b	1.3	76.4-86.7	99.7 ^a	0.2	98.3-100.0
14:0	99.0 ^a	0.2	98.3-100.0	79.2 ^b	1.2	75.4-85.4	99.4 ^a	0.2	98.2-100.0
16:0	94.7 ^a	0.6	92.7-97.6	87.8 ^b	0.6	85.6-91.2	96.4 ^c	0.1	95.8-96.9
18:0	86.3 ^{ab}	2.2	75.2-96.8	84.0 ^a	2.2	74.4-92.1	91.1 ^b	1.0	85.5-96.2
¹ MUFA: 16:1	99.6 ^a	0.4	96.2-100.0	100.0 ^a	0	100.0-100.0	100.0 ^a	0	100.0-100.0
18:1	94.3 ^{ab}	0.6	92.5-96.9	93.1 ^a	1.0	87.1-96.4	95.9 ^b	0.2	94.7-97.1
¹ PUFA: 18:2	94.2 ^a	1.3	88.3-98.6	92.2 ^a	2.2	77.6-97.7	96.7 ^a	0.5	94.5-98.7
18:3	93.2 ^a	0.8	88.7-96.5	96.8 ^b	0.6	93.2-98.8	97.7 ^b	0.4	95.8-99.3

^{a,b,c} Means with different superscripts within the same row were significantly ($P < 0.05$) different.

*SE: Standard error.

¹SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Table 2.9. Models used for predicting metabolisable energy values (KJ/g) of the experimental diets.

	Reference	Model
<u>Factorial Model</u>		
Atwater, (1910)	(Atwater, 1910)	$ME_{\text{Atwater}} = 16.7P + 37.6F + 16.7C$
British approach, (1991)	(Holland <i>et al.</i> , 1991)	$ME_{\text{British}} = 17P + 37F + 16Cm$
FDA, (1993)	(FDA, 1993)	$ME_{\text{FDA}} = 16.7P + 37.6F + 16.7(C - \text{isDF})$
<u>Empirical model</u>		
Levy <i>et al.</i> , (1958)	(Levy <i>et al.</i> , 1958)	$ME_{\text{Levy}} = 0.976E - 33.3N - 250$
Miller and Payne, (1959)	(Miller & Payne, 1959)	$ME_{\text{M\&P}} = 0.95E - 31.4N$
Southgate, (1975)	(Southgate, 1975)	$ME_{\text{Southgate}} = 0.977E - 16.7UC - 27.6N$
Miller and Judd, (1984)	(Miller & Judd, 1984)	$ME_{\text{M\&J}} = (0.95E - \text{DF}\%) - 31.4N$
Livesey, (1991)	(Livesey, 1991a)	$ME_{\text{Livesey}} = 0.96E - 8.4U - 30N$

*ME(KJ/g): metabolisable energy; P(g): dietary protein, F(g): dietary Fat; C(g): total carbohydrate by difference; isDF(g): insoluble dietary fibre; Cm(g): determined available carbohydrate expressed as equivalent weight of monosaccharide; E(kJ/g): gross energy of diet; N(g): dietary nitrogen; DF%: total dietary fibre as a percentage of the dry weight of the diet; UC(g): unavailable carbohydrate determined as non-starch polysaccharide; U(g): unavailable complex carbohydrate; FDA: Food and Drug Administration; British approach: Holland *et al* (1991).*

Table 2.10. Mean (n=9) determined metabolisable energy (ME) values, predicted ME values based on the application of different models and differences between determined ME and predicted ME for the three experimental diets.

	Refined Diet			Fruit & Vegetable Diet			Cereal Diet		
	Mean	SE	Range	Mean	SE	Range	Mean	SE	Range
Determined GE (kJ/gDM diet)	23.397 ^a	0.03	23.189-23.519	19.741 ^b	0.02	19.710-19.903	19.840 ^c	0.01	19.782-19.877
Determined ME (kJ/gDM diet)	21.111 ^a	0.06	20.861-21.445	17.043 ^b	0.14	16.357-17.847	16.814 ^b	0.05	16.559-17.101
	ME value		Diff.%	ME value		Diff.%	ME value		Diff.%
<u>Predicted ME using factorial models (KJ/g DM diet)</u>									
ME _{Atwater}	21.884		3.7**	18.873		10.8***	18.274		8.3***
ME _{FDA}	21.134		0.1 ^{NS}	17.738		4.1***	16.616		-1.2**
ME _{British}	21.057		-0.3 ^{NS}	16.809		-1.3 ^{NS}	16.122		-4.1***
<u>Predicted ME using empirical models (KJ/g DM diet)</u>									
ME _{Levy}	21.398		1.4**	18.084		6.2***	17.966		6.9***
ME _{M&P}	21.344		1.1**	18.096		6.2***	18.052		7.4***
ME _{M&J}	21.295		0.9*	18.018		5.8***	18.030		7.2***
ME _{Southgate}	21.486		1.8***	17.761		4.3***	17.378		3.4***
ME _{Livesey}	21.211		0.5 ^{NS}	17.661		3.7***	17.445		3.8***

$Diff.\% = (ME\ predicted - ME\ determined) / ME\ determined \times 100$

^{abc} Means with different superscripts within the same row were significantly ($P < 0.001$) different.

SE: Standard error.

NS=Non significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, for significance of difference between predicted and determined values.

2.7 Discussion

The study sought to determine the degree of variation in the apparent faecal digestibility of protein, fat and carbohydrate (by difference) and urinary energy excretion, and also the predictive accuracy of standard Atwater factors, for a range of complex mixed diets typical of those consumed by humans. The diets were formulated, therefore, using food ingredients commonly eaten in western society and the diets ranged from low fibre, high fat (3.5 gTDF/100g dry matter; 27.6 gFat/100g dry matter) to high fibre, low fat (7.8 gTDF/100g dry matter; 10.9 gFat/100g dry matter) diets. As would be expected with such variation in ingredient and nutrient composition, the determined dietary ME values varied considerably (16.81, cereal to 21.1, refined kJ/g dry matter).

Dietary ME was determined by carefully measuring faecal and urinary energy losses for the subjects and subtracting these from food energy eaten. No account was taken of the loss of energy via breath and flatus mainly in the form of hydrogen and methane gases. Correction for gaseous energy loss would have led to somewhat lower determined ME values (though the gaseous energy losses are generally regarded as being small; 1% of ingested ME (Blaxter, 1989; Poppitt *et al.*, 1996)), and an even greater difference between Atwater predicted and determined ME values. Also, in assessing the Atwater derived ME values it is most appropriate to use the traditional ME system (where gaseous and surface energy losses are considered to be negligible), as the Atwater factors are based on such a system and purport to predict ME according to this definition.

There are a number of important shortcomings of Atwater factors. Firstly, the gross energy contents of protein, fat or carbohydrate are not constants. The heats of combustion of fats, for example, range from 34 KJ/g for medium-chain triacylglycerol to up to 39.7 KJ/g for long-chain triacylglycerol and the heat of combustion of food proteins range from 21 to 25 KJ/g and 66 to 152 KJ/g nitrogen (Livesey, 1995b). Asparagin, representative of the non-protein nitrogen fraction of vegetables, contains only 14.43 KJ/g (Merrill & Watt, 1973). Secondly, there are chemical components present in foods other than protein, fat and available carbohydrate that contribute energy and that may influence the ME of the principal chemical components. In particular, the influence of dietary fibre needs to be accounted for. Developments in analytical methodology also raise questions, such as how

should dietary carbohydrate be defined? How should dietary fibre be determined? What factors should be used to convert nitrogen to protein? Which solvents should be used to extract dietary fat? Thirdly and as highlighted by the present results, the digestibility of the principal chemical components, protein, fat and carbohydrate, may vary quite considerably among diverse diets and foods, as does the urinary energy per unit urinary nitrogen. Merrill and Watt (1973) discussed the wide variation in observed digestibility around the average digestibility values adopted by Atwater (protein 92, fat 95 and carbohydrate 97%) and the high degree of variation observed for the urinary energy: nitrogen ratio, and questioned the use of an average urinary energy value, especially when applied to high-protein diets. All of these sources of variation mean that Atwater factors may at best be only an approximation. Particularly, where diets or specific foods contain novel ingredients (e.g. Polydextrose®, sugar alcohols, maltodextrins, oligosaccharides, resistant starch, caprenin) that have atypical digestibility and metabolisability, the Atwater factors may be quite misleading (Livesey, 1993; Matthews, 1995).

In the present study where diverse complex mixed diets were consumed by human subjects, the Atwater system overestimated mean determined ME by up to 11%. This is consistent with the findings of other studies that unmodified Atwater factors lead to inaccuracy in the prediction of dietary ME, (Merrill & Watt, 1973; Southgate & Durnin, 1970; Livesey, 1990). The two modified factorial models investigated here, mainly differing in the way in which the carbohydrate component is determined (FDA (1993): carbohydrate determined by difference and corrected for insoluble dietary fibre; British (Holland *et al.*, 1991): carbohydrate determined as available carbohydrate expressed as monosaccharide equivalents), gave better predictive accuracy but differences between predicted and determined ME values of up to 4% remained. The FDA and British models assume that unavailable carbohydrate has no nutritive value and the factors for protein, fat and available carbohydrate are completely independent of one another. Both of these assumptions are untenable (Blaxter, 1989). Some of the 'unavailable' carbohydrates are fermented by the microbiota of the large intestine, and short chain fatty acids (SCFA) are produced. SCFA can be absorbed and used by the host as an energy source. Also an increase in the dietary unavailable carbohydrate component may depress the apparent digestion of protein, fat and available carbohydrate.

That there is considerable variation in nutrient digestibility and urinary energy excretion, even for conventional food ingredients, which would be heightened further with the inclusion of novel food ingredients, can be capitalised upon to develop specific lower ME weight-loss foods for humans. By way of example and for the results reported here, the apparent faecal digestibility of energy was 5 percentage units lower for the cereal versus refined diet, the digestibility of protein was 9 percentage units lower for the fruit and vegetable versus refined diet and the digestibility of fat was 9 percentage units lower for the fruit and vegetable versus refined diet. The digestibility of C14:0 fatty acids was 20 percentage units lower for the fruit and vegetable diet versus cereal or refined diet. Similarly, there was quite some variability in urinary energy excretion. The excretion of urine energy per unit urine nitrogen (KJ/gN) was 10 units higher for the fruit and vegetable versus refined diet. The energy : nitrogen ratio of urine ranged from 32.5 to 47.7 KJ/gN among individual subjects on the three diets. The mean values for the urinary energy: nitrogen ratio were statistically significantly ($P < 0.001$) different from the classic value reported by Atwater (33.4 KJ/g urinary N) except for the refined diet.

In developing and evaluating specialised low-calorie foods, where quite small differences in ME may be of practical significance, the use of Atwater or modified Atwater factors is likely to be too inaccurate. A validated rapid and more direct means of determining ME in foods, perhaps based on the use of model laboratory animals and *in vitro* digestion methods would be of considerable value in the development of such specialised foods.

In general the empirical models applied in the present study were no more accurate than the modified Atwater factorial models. Brown *et al* (1998) have reviewed other relevant published comparisons, which accord with our findings. Application of an empirically based equation developed by Brown *et al.* (1993) (results not given) did lead to a rather accurate prediction of ME. However, the Brown *et al.* (1993) equation accounts for energy lost in converting amino N to urea (cost of urea synthesis), which is not accounted for in the determined ME values, making a direct comparison difficult. Livesey (1990) derived a modified Atwater factorial model which included an added factor for dietary fibre. When this model was applied in the present study good agreement between predicted and observed ME was found for the high fibre diets, but there was a 2% unit difference for the refined diet. When the Livesey's modified Atwater factorial model was applied by Brown *et al.* (1998) to the data previously published by Baer *et al.* (1997) a positive bias (3-7%) was

found for very low fat diets. In our own laboratory (Coles LT and Moughan PJ, unpublished data) we have found an underestimation (4%) of determined ME when the Livesey's modified Atwater model was applied to a high pectin containing diet. In general, the empirical models were more accurate for the refined diet than the high fibre diets. Brown *et al* (1998) also found that several general energy assessment systems were inaccurate for predicting ME for high NSP diets. Livesey (1995b) has discussed the shortcomings of the factorial approach and the advantages of using empirical models based on determined gross energy and predictors of energy excretion. A remaining inherent shortcoming of statistical models, however, concerns the possibility of errors arising when extrapolation occurs beyond the boundaries of the original experimental dataset (Livesey, 1995b). This may be a particular concern when such models are applied to novel foods having more extreme chemical compositions. It is concluded that some of the empirical models evaluated here and the modified Atwater factors may be suitably accurate ($\pm 5\%$) for use in general dietary labelling and for determining dietary ME intakes of groups and populations dependent upon the level of accuracy considered acceptable, but will be less useful where a higher degree of accuracy is required.

A concept of net metabolizable energy (NME) has been discussed for deriving food energy values (Livesey *et al.*, 2000). NME attempts to reflect the food energy that can be converted to ATP energy within the body, by deducting obligatory thermogenesis from ME. The NME system provides for a more accurate assessment of the 'available' energy content of foods and food ingredients, and a better basis of comparison among diverse foods and food ingredients. Application of the NME system for food labeling purposes has been hampered, however, based largely on pragmatic arguments (Warwick & Baines, 2000).

For an accurate evaluation of the 'available' energy content of food ingredients and especially novel formulated weight-loss foods, it may be worthwhile to determine digested and fermented nutrients (determination at both ileal and faecal levels), predict using stoichiometric relationships potential ATP production, predict urinary energy losses, and thus a net metabolizable energy value. The different absorbed nutrients (e.g. amino acids, fatty acids, glucose, volatile fatty acids) are used for ATP production with different biochemical efficiencies. With such a system, based on nutrient uptake, the losses of energy in gases and as heat of fermentation are predicted based on the determined loss of material in the colon arising due to bacterial fermentation.

Chapter 3 General Summary and Discussion

The study reported herein has tested the accuracy of current food energy evaluation systems (three factorial systems and five empirical models) on three diets varying quite widely in dietary fibre and fat contents by comparing calculated metabolizable energy (ME) values with determined ME. The results demonstrate that Atwater factors lead to a statistically significant overestimation of metabolizable energy content. The FDA factors and British modified Atwater factors gave a better estimation by taking into account the influences of dietary fibre, but practically important differences (4%) remained. The empirical models evaluated were found to be accurate to within ~ 2% for the Refined diet (high fat, low fibre), but a positive bias (3.4 – 7.4%) was found for both the Fruit and Vegetable and Cereal diets (high fibre, low fat). In general, the empirical models did not lead to a higher level of accuracy of prediction compared to the factorial models. The Atwater specific factors, often regarded as the gold standard for food energy assessment in the U.S. (Merrill & Watt, 1973; Livesey, 1990; Mathews, 1995), were not tested in the present study because the errors arising from the estimation of the macronutrient contents in each food ingredient, especially those for pre-made foods (e.g. mince, cheese pie, chocolate bar, muffin), may have overwhelmed the true differences between predicted and determined ME values. Studies reported by Brown *et al* (1998) showed that the Atwater specific factors gave up to 6% bias on the prediction of ME for high NSP diets. Overall, some of the empirical models evaluated here and the modified Atwater factors may be suitably accurate ($\pm 5\%$) for use in general dietary labelling and for determining dietary ME intakes of groups and populations dependent upon the level of accuracy considered acceptable, but will be less useful where a higher degree of accuracy is required, especially when low calorie foods, novel food ingredients and special formulated weight-loss diets are involved.

In the presently reported study, summation of ME values sourced from published food composition tables underestimated the metabolizable energy values by 4% for the Refined diet and 23% for the Fruit and Vegetable and Cereal diets. Such discrepancies are a natural consequence of food composition differences between the food ingested and the average tabulated values. Therefore, it is important to bear in mind that food composition tables report 'representative' values and exact correspondence with selected food items should not be expected. In situations whereby precise data on the energy values of diets is required,

such as specially formulated diets for burn patients, diabetics or people willing to control body weight, prediction of metabolizable energy value based on determined composition data is a better alternative.

In the work reported here, determined ME was 0.90 determined GE for the refined diet, 0.86 determined GE for the fruit and vegetable diet and 0.85 determined GE for the cereal diet. This demonstrates that gross energy is the major contributor to dietary metabolizable energy. The latter important observation was noted previously by Southgate and Durnin (1970) who suggested that ‘calculations of metabolizable energy would probably be best improved by devoting more attention to the gross energy content of different foodstuffs rather than by attention to the ‘digestibility’ of the different components of foods.’ Allison and Senti (1983) also recommended in their review concerning ME that measurement of gross energy is a high priority in improving the adequacy of food databases. The only truly accurate measure of energy content among current energy evaluation systems (both factorial and empirical approaches) is the heat of combustion (gross energy) of a food and all other measures derived from experimental studies are approximations (BNF, 1990). Therefore, development of a reference database reporting the gross energy values of individual foods, especially those foods (such as food substitutes, sweeteners, bulking agents) that do not fit the current food classes, would be useful. When sufficient data become available on the gross energy contents of a wide range of foods, predicting metabolizable energy value based on gross energy content may be more appropriate. This may be especially so for foods designed for medical purposes and foods containing novel ingredients.

The present results demonstrate that there is considerable variation in nutrient digestibility among diverse diets. **Table 3.1** lists average apparent faecal digestibility for protein, fat and “total carbohydrate (CHO) by difference” for the three experimental diets along with Atwater’s average coefficients of digestibility. Apparent digestibility of protein was significantly lower in the fruit & vegetable and cereal diets compared to Atwater’s values. The apparent digestibility of fat was significantly lower for the fruit & vegetable diet, and the apparent digestibility of total carbohydrate by difference was significantly lower in the cereal diet. When Atwater estimated coefficients of digestibility for the protein, fat and carbohydrate components of individual foods, based on results of many digestion experiments, he found that digestibility was significantly different for the nutrients in

different food groups (Merrill & Watt, 1973). Therefore, Atwater developed his average coefficients of digestibility for nutrients in a mixed diet using a weighted diet composition based on a consumption statistics survey of 185 dietaries, to mimic the diet most common at that time.

Table 3.1. Mean apparent faecal digestibility of protein, fat and total carbohydrate for three experimental diets (present study) and Atwater's average coefficient of digestibility.

	Atwater's value	Refined Diet	Fruit & Vegetable Diet	Cereal Diet
Digestibility of protein %	92	90.0	81.4	82.7
Digestibility of fat %	95	95.7	87.0	95.1
Digestibility of total CHO* %	97	94.4	95.5	91.1

* total CHO = 100 - % Moisture - % Protein - % Fat - % Ash

Adjustment can be made for these average digestibility coefficients on the basis of the proportions of various food sources represented in a particular diet (Bernstein *et al.*, 1955). However, it is difficult to predict the supplemental action of one food upon another in a complex diet on the digestibility coefficients of the nutrients. Early studies already showed that milk and bread are more assimilated when fed together than when given separately (Woods & Merrill, 1900; Bryant, 1897). Others reported that dietary fibre and dietary calcium depress digestibility more in beef tallow supplemented diets, than with vegetable or fish oil supplemented diets (Denke *et al.*, 1993; Fang & Kies, 1993; Hanson *et al.*, 1993). Synergistic effects in the digestibility of fats are well documented. Results from our study showed that the increased dietary fibre intake in both the Fruit & Vegetable and Cereal diets led to a significant decrease in the digestibility of protein, regardless of the source of fibre. The effect of fibre intake on the digestibility of fat, however, was dependent on the source of fibre. Greater fat / fatty acid losses in faeces occurred only with the Fruit & Vegetable diet. Many other studies (Kelsay, 1978) have shown that different forms of fibre have different impacts on body functions. Apples, apricots and prunes can increase the volume of stools and amounts of volatile fatty acids (Olmsted *et al.*, 1934); wheat fibre (containing largely cellulose and hemicellulose) alters colonic functions by decreasing transit time, increasing faecal weight and the number of defecations. Pectin lowers blood lipid levels and increases faecal lipids and bile acids (Kay & Truswell, 1977). Therefore, specialized diets, such as weight loss diets, diabetic control diets or cholesterol reducing diets can be formulated based on the detailed and precise information of impacts of different food ingredients on nutrient digestibility and physiological effects. A validated

rapid and more direct means of determining the digestibility of nutrients in different diets, perhaps based on the use of laboratory animal models and *in vitro* digestion methods would appear to be of considerable value to fulfil this purpose.

For the three diets studied here the apparent digestibility of energy was significantly lower for the high dietary fibre diets (both Fruit & Vegetable and Cereal diets). An increased faecal excretion of nitrogen and fat in addition to that of dietary fibre contributed to greater faecal energy losses. The fruit fibre had a notable impact on the apparent digestibility of fat and fatty acids, especially for the saturated fatty acids (Refer to Table 2.8). How dietary fibre affects the digestion of fatty acids in humans is not completely understood, the low digestibility of fatty acids observed in the Fruit and Vegetable diet may be caused by the fermentation process in the large intestine, whereby C12:0 and C14:0 may be synthesised microbially and C16:0 can be hydrogenated microbially from C16:1 (Livesey, 2000). N retention and excretion were influenced by both the level and source of dietary fibre (Table 3.2). N retention was considerably reduced with high fibre diets (both the Cereal diet and the Fruit & Vegetable diet), but the reduction reached statistical significance only for the Cereal diet. Faecal N excretion was statistically significantly higher with the Fruit and Vegetable (1.86 g/g N intake) and Cereal diet (1.73 g/g N intake) compared to the Refined diet (1.00 g/g N intake). A reduction in urinary N excretion occurred with the Fruit and Vegetable diet (0.792 g/g N intake compared to 0.90 g/g N intake for the Cereal diet). Faecal N excretion consists mainly of bacterial protein, secreted digestive enzymes, and

Table 3.2. Mean daily nitrogen (N) intakes, retention and excretions for the subjects receiving the three experimental diets.

	Refined Diet	Fruit & Vegetable Diet	Cereal Diet
N intake (g/day)	14.74 ^a	11.63 ^a	12.45 ^a
N retention * (g/g N intake)	0.109 ^a	0.022 ^{ab}	-0.073 ^b
Urinary N excretion (g/g N intake)	0.792 ^a	0.792 ^{ab}	0.900 ^b
Faecal N excretion (g/g N intake)	0.100 ^a	0.186 ^b	0.173 ^b

* $N \text{ retention} = N_{\text{intake}} - (N_{\text{urine}} + N_{\text{faeces}})$

^{a,b} Means with different superscripts within the same row were significantly ($P < 0.05$) different.

sloughed mucosal cells (Mason, 1984; Eggum, 1992; Tetens *et al.*, 1996). The increased dietary fibre intake for subjects on the Fruit and Vegetable and Cereal diets may lead to an increase in the flow of substrate to the large intestine, thus stimulating bacterial growth

resulting in an increase of faecal N excretion. The N used for bacterial growth is mainly derived from NH₃ in the large intestine (Wrong & Vince, 1984), which in turn is mainly derived from endogenous urea (Rémésy & Demigné, 1989). A decrease in urinary N excretion observed with the Fruit & Vegetable diet may, therefore, be in compensation for the increased faecal N excretion. The similarity of faecal N excretion between the two high fibre diets (Fruit and Vegetable, and Cereal diet) and that faecal bulking increased with dietary fibre intake (108.9 versus 82.3 mg dry faecal weight / g DM intake for the cereal and fruit and vegetable diet, respectively) (Table 3.3) suggests that the dietary fibre in the Cereal diet was less fermentable than the fibre in the Fruit and Vegetable diet. Poorly fermentable fibre passes through the large intestine less changed, leading to an increase in faecal bulking, and resulting in a dilution of faecal N. In contrast, highly fermentable fibre stimulates bacterial growth, leading to a reduction in the amount of fibre residue, and resulting in a N enrichment in faecal excretion. It might be possible to predict the N excretion pattern based on the fermentability of the dietary fibre source in the diet. A reduction of urinary N excretion due to high dietary fibre intake may be beneficial in certain clinical situations (i.e. patients with liver or renal failure).

Table 3.3. Mean daily dietary fibre (TDF) intakes and faecal excretions expressed as both wet and dry weight for the subjects receiving the three experimental diets.

	Refined Diet	Fruit & Vegetable Diet	Cereal Diet
TDF intake (g/day)	25.19 ^a	43.79 ^b	49.21 ^b
Faecal weight (g dry weight/day)	34.78 ^a	46.83 ^{ab}	53.65 ^b
Faecal weight (g wet weight/day)	136.63 ^a	257.85 ^{ab}	276.63 ^b
Faecal bulking (g wet weight/g DM intake)	257.6 ^a	440.4 ^{ab}	559.8 ^b
Faecal bulking (g dry weight/g DM intake)	66.2 ^a	82.3 ^a	108.9 ^b

^{a,b,c} Means with different superscripts within the same row were significantly ($P < 0.05$) different.

Atwater used an average value of 7.9 kcal / g of urinary nitrogen to estimate the available energy content of protein, which corresponds to about 1.25 kcal / g of protein absorbed (ingested less digestive loss), to correct for the energy loss of incompletely oxidized material in the urine. This was based on the assumption that there was body N balance and that all of the non-metabolized part of the available N was recovered in the urine. The calorie-nitrogen ratio of the urine can be affected by a number of factors such as level of food intake, extent of digestibility, type of diet, and degree of physical activity. Results

from our study showed that the urinary calorie-nitrogen ratio was 8.10, 10.54 and 8.99 for the Refined diet, Fruit & Vegetable diet and Cereal diet, respectively. Many other studies have also reported a wide variation in the calorie-nitrogen ratio on different foods, especially under fasting conditions (Benedict, 1907; 1915; Southgate & Durnin, 1970). Therefore, use of Atwater's mean calorie-nitrogen value of 7.9 kcal/g N to predict the urinary energy for individuals compromises accuracy, but for practical purposes the differences between the urinary energy loss calculated using this mean ratio and the observed urinary energy loss are small compared with the gross energy intake. The error introduced by using Atwater's average calorie-nitrogen value does not greatly affect on the calculated available energy value of the whole diet.

As early as 1885 Rubner had recognized that chemical methods for determining the energy-yielding components in a food were not entirely satisfactory. Innumerable improvements in methods and techniques for separating and determining the fractions of energy-yielding components have been made since then. With current knowledge on food composition each of the energy-yielding food constituents can be broken down into a variety of subfractions or components. For example, protein content can be expressed as the sum of the amino acids, which requires no assumption about, or knowledge of, either the non-protein nitrogen content of the food or the relative proportions of specific amino acids, which was the problem with the use of total N combined with a conversion factor. Similarly, total fat can be expressed as triglyceride equivalents determined as the sum of individual fatty acids and expressed as triglycerides (FAO, 1994). Until now the most common method for measuring fat content in a food was the gravimetric extraction method. Fat content of foods determined by extraction may vary depending on the solvent used. A solvent with more polarity such as chloroform-methanol will give a more complete extraction compared to ether, which was used by Atwater. Therefore, the heats of combustion of the extracted lipid reported by Atwater may not be adequate for the extracted lipid as currently determined (Miles *et al.*, 1981). For energy purposes, the adoption of the definition of fat as the sum of fatty acids expressed as triglyceride equivalents, leads to the possibility of assigning individual heat of combustion values to each component.

A feature that distinguishes the Atwater system from other systems for estimating metabolizable energy is the measurement of carbohydrate by difference. Although carbohydrate by difference does not give detailed information on the individual

carbohydrate components, it does provide the Atwater system with an inherent robustness. Errors in protein and fat determination can be compensated for or partially compensated for by inverse changes in the content of carbohydrate. However, information on the carbohydrate content of foods such as that obtained by direct analysis in the U.K. system has applications beyond the calculation of ME. Carbohydrate content determined by direct analysis will be able to give detailed information on the individual carbohydrate components. Modern analytical methods allow an accurate determination of all of the major energy-containing compounds present in a food. Laboratory animal models and in vitro digestion and fermentation systems, moreover, allow prediction of nutrient uptake from the gut and short chain fatty acid and gas production in the colon. Combining these approaches it is possible to predict the uptake of individual food nutrients, and by applying stoichiometric relationships, ATP production and urinary energy losses. Development of such a system, based on nutrient uptake, the losses of energy in gases and as heat of fermentation, and the loss of material arising due to bacterial fermentation (at both ileal and faecal levels) may be worthwhile for an accurate evaluation of the 'available' energy content of food ingredients and especially novel formulated weight-loss foods.

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APPENDICES

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Appendix 1.1. Physical characteristics of the subjects and their daily energy intake level.

Participants	ID Code	Sex	Age (yrs)	Diet	Intake Level	Height (cm)	Weight (kg) before study	Weight (kg) after study
1	TD0101	M	37	Refined	2.5	175.4	71.3	71.3
2	TD0201	M	31	Refined	3	165.5	64.85	65
3	TD0402	F	37	Refined	1.5	160.8	67.9	67.3
4	TD0502	F	46	Refined	2	161.5	65.95	65
5	TD0601	M	26	Refined	2	176	75.7	75.5
6	TD0701	M	36	Refined	2	188.1	81.7	81.7
7	TD0802	F	36	Refined	1.5	166.5	54.3	52.7
8	TD0902	F	30	Refined	1.5	155.2	58.6	57.5
9	TD1002	F	44	Refined	2	169.8	120.4	120
10	FV0102	F	34	Fruit & Veg.	1.5	161.5	52.2	50.4
11	FV0301	M	26	Fruit & Veg.	2	179	123	123
12	FV0401	M	44	Fruit & Veg.	2	168.5	87.8	86.2
13	FV0502	F	59	Fruit & Veg.	1	155.4	71.75	71
14	FV0602	F	35	Fruit & Veg.	1.5	172	59.8	59.2
15	FV0702	F	24	Fruit & Veg.	1	160	55	53.8
16	FV0802	F	39	Fruit & Veg.	2	171.8	84.1	84
17	FV0901	M	27	Fruit & Veg.	2	172.5	72.5	71.9
18	FV1001	M	29	Fruit & Veg.	2	172	76.2	76.2
19	CM0102	F	47	Cereal	1	177.8	87.2	86.7
20	CM0202	F	39	Cereal	1	157	75	74
21	CM0301	M	48	Cereal	2	177.6	91.6	90.5
22	CM0401	M	26	Cereal	1	179.3	67.2	66.8
23	CM0502	F	52	Cereal	1	163.6	77.8	76.9
24	CM0602	F	23	Cereal	1	154	45.8	45.5
25	CM0701	M	27	Cereal	1.5	176	74	72.5
26	CM0802	F	35	Cereal	1	161.5	53	50
27	CM0901	M	52	Cereal	2	180	69.4	68.7

Appendix 1.2. Individuals' energy and mass of intake and excretion for the three experimental diets.

		Intake MJ/day	Faeces MJ/day	Urine MJ/day	Intake gDM/day	Intake g fresh/day	Faeces gDM/day	Faeces gFresh/day	DE MJ/day	ME MJ/day
Refined Diet	TD0101	15.990	1.086	0.468	684.3	1590.8	48.10	178.7	14.904	14.436
	TD0201	16.790	0.992	0.400	718.0	1654.3	47.44	242.0	15.798	15.398
	TD0402	9.365	0.549	0.349	403.9	953.5	23.23	85.3	8.816	8.467
	TD0502	11.995	0.825	0.350	510.0	1272.8	34.67	113.9	11.170	10.820
	TD0601	12.784	0.972	0.446	544.9	1271.9	42.49	199.1	11.813	11.367
	TD0701	12.791	0.748	0.419	545.2	1272.5	29.70	89.0	12.042	11.623
	TD0802	9.056	0.590	0.281	386.9	954.5	26.96	140.3	8.467	8.185
	TD0902	9.076	0.615	0.328	387.8	956.6	26.26	76.2	8.461	8.133
	TD1002	12.402	0.830	0.449	530.5	1272.0	34.20	105.1	11.572	11.123
Fruit&Veg. Diet	FV0102	9.811	0.955	0.361	497.4	1123.2	39.21	169.5	8.857	8.496
	FV0301	13.070	1.903	0.331	662.5	1490.8	83.10	607.1	11.167	10.836
	FV0401	13.075	1.641	0.439	662.8	1491.4	70.95	531.5	11.434	10.995
	FV0502	6.608	0.529	0.287	335.3	753.2	21.89	84.8	6.079	5.793
	FV0602	10.195	0.673	0.380	512.3	1121.8	27.51	110.5	9.522	9.142
	FV0702	6.570	0.650	0.298	333.2	754.5	27.53	138.1	5.919	5.621
	FV0802	13.089	1.311	0.469	663.5	1490.3	54.90	256.5	11.778	11.309
	FV0901	13.030	1.132	0.497	660.4	1490.4	44.86	178.1	11.898	11.402
	FV1001	13.200	1.273	0.494	670.1	1491.3	51.55	244.5	11.927	11.433
Cereal Diet	CM0102	7.302	0.795	0.369	367.9	1080.9	38.62	197.9	6.507	6.138
	CM0202	7.258	0.809	0.318	365.1	1080.9	40.66	162.1	6.448	6.130
	CM0301	14.610	1.580	0.651	736.1	2162.1	79.93	403.1	13.031	12.379
	CM0401	11.184	1.142	0.465	565.3	1621.2	58.06	264.5	10.042	9.576
	CM0502	7.271	0.755	0.350	366.3	1081.6	37.34	179.1	6.515	6.165
	CM0602	7.243	0.723	0.264	365.6	1081.5	36.84	200.5	6.520	6.256
	CM0701	10.961	1.275	0.455	552.3	1622.0	63.93	349.3	9.686	9.231
	CM0802	7.325	0.884	0.330	369.1	1083.8	44.96	277.9	6.441	6.111
	CM0901	14.625	1.637	0.548	736.8	2164.2	82.48	455.2	12.987	12.440

Appendix 1.3. Individuals' intake and excretion of macronutrients for the three experimental diets.

		Fat intake g/day	Protein intake g/day	total CHO intake g/day	avaCHO as weight gDM/day	avaCHO as mono-equiv gDM/day	TDF intake gDM/day	IDF intake gDM/day	NSP intake gDM/day	Faecal CHO g/day	Faecal Fat g/day
Refined Diet	TD0101	186.79	121.41	354.15	314.54	338.35	32.74	30.80	25.34	23.19	8.70
	TD0201	194.25	126.24	374.71	333.59	358.68	34.03	32.03	26.33	26.44	5.32
	TD0402	105.73	70.74	214.46	191.02	205.39	19.19	17.93	14.97	8.39	4.69
	TD0502	141.33	89.26	263.17	235.84	253.43	23.16	22.10	18.30	13.30	6.69
	TD0601	149.33	96.60	281.61	251.44	270.48	25.78	24.56	19.76	19.12	7.64
	TD0701	149.41	96.65	281.75	251.57	270.61	25.79	24.57	19.77	13.06	5.69
	TD0802	107.51	67.79	199.08	176.39	189.89	19.63	17.71	13.16	12.78	4.03
	TD0902	107.74	67.94	199.51	176.77	190.30	19.67	17.74	13.19	8.38	6.14
	TD1002	146.97	92.17	274.06	242.93	261.65	26.73	23.97	18.06	14.62	5.56
Fruit&Veg. Diet	FV0102	65.21	65.18	350.80	290.05	303.60	39.33	34.03	28.09	14.02	7.70
	FV0301	86.72	86.91	467.21	386.14	404.26	52.35	45.31	37.81	41.68	15.88
	FV0401	86.76	86.94	467.41	386.31	404.44	52.37	45.33	37.83	30.10	13.99
	FV0502	43.36	44.02	236.68	194.59	203.66	26.68	22.69	19.39	7.77	4.53
	FV0602	65.00	65.64	365.07	302.66	316.84	39.64	33.93	28.63	9.60	5.48
	FV0702	43.50	43.53	235.26	194.92	204.01	26.30	22.72	18.82	10.42	5.11
	FV0802	86.77	87.29	467.67	384.76	402.76	52.72	45.31	37.93	20.74	10.49
	FV0901	86.62	86.66	465.54	384.66	402.65	52.10	45.08	37.25	14.15	11.68
		FV1001	86.69	87.30	474.27	391.46	409.79	52.63	45.20	37.90	17.90
Cereal Diet	CM0102	40.13	58.26	254.16	202.93	216.26	36.85	36.58	28.77	20.20	2.06
	CM0202	40.17	58.34	251.22	199.76	212.94	37.06	36.80	28.86	22.16	2.66
	CM0301	80.28	116.56	508.52	405.95	432.62	73.79	73.25	57.59	49.29	4.26
	CM0401	60.21	87.97	393.85	317.41	338.05	55.77	54.95	43.79	32.07	1.88
	CM0502	39.99	58.08	252.96	201.92	215.16	36.62	36.39	28.56	20.58	2.20
	CM0602	39.00	58.17	253.34	201.53	214.66	36.56	36.15	28.54	21.96	1.46
	CM0701	60.23	87.45	381.52	304.56	324.57	55.37	54.97	43.21	36.70	3.00
	CM0802	40.25	58.44	254.96	203.52	216.89	37.02	36.76	28.88	25.68	1.95
		CM0901	80.36	116.68	509.02	406.36	433.05	73.86	73.33	57.64	46.80

Appendix 1.4. Individuals' intake and excretion of nitrogen and urinary nitrogenous constituents.

		Intake N (g/day)	Feacal N (g/day)	Urinary N (g/day)	creatinineN (g/day)	ammoniaN (g/day)	uric acid N (g/day)	urea N (g/day)	urine Energy:urine N kJ/gN	urine Energy:N intake kJ/gN
Refined Diet	TD0101	19.443	1.782	14.325	0.472	0.536	0.095	12.646	32.67	24.07
	TD0201	20.215	1.873	12.143	0.489	0.442	0.097	10.648	32.93	19.78
	TD0402	11.334	1.136	10.066	0.372	0.377	0.065	8.745	34.63	30.76
	TD0502	14.282	1.602	10.231	0.337	0.370	0.071	8.799	34.20	24.50
	TD0601	15.456	1.787	13.726	0.572	0.513	0.096	11.994	32.51	28.87
	TD0701	15.464	1.141	12.410	0.453	0.492	0.063	10.816	33.80	27.12
	TD0802	10.847	1.121	8.263	0.255	0.352	0.078	7.142	34.07	25.95
	TD0902	10.871	1.215	9.454	0.287	0.389	0.139	8.451	34.65	30.14
	TD1002	14.749	1.425	12.693	0.379	0.407	0.114	11.104	35.36	30.43
Fruit&Veg. Diet	FV0102	10.428	2.091	7.980	0.351	0.247	0.059	7.530	45.23	34.61
	FV0301	13.905	2.791	8.457	0.462	0.430	0.056	7.104	39.13	23.79
	FV0401	13.911	3.030	10.130	0.442	0.529	0.055	8.163	43.32	31.55
	FV0502	7.060	1.107	6.434	0.260	0.256	0.067	5.494	44.54	40.59
	FV0602	10.519	1.512	8.148	0.337	0.299	0.075	6.909	46.64	36.13
	FV0702	6.965	1.483	7.293	0.334	0.267	0.047	7.007	40.92	42.85
	FV0802	13.983	2.906	9.827	0.369	0.281	0.067	7.987	47.69	33.52
	FV0901	13.865	2.191	11.250	0.498	0.475	0.074	9.240	44.15	35.82
	FV1001	13.985	2.477	10.899	0.471	0.409	0.129	8.746	45.31	35.31
	Cereal Diet	CM0102	9.321	1.832	9.430	0.359	0.280	0.104	7.851	39.11
CM0202		9.334	1.650	8.194	0.347	0.320	0.126	6.739	38.87	34.12
CM0301		18.650	2.471	17.631	0.518	0.729	0.220	14.929	36.95	34.93
CM0401		14.092	2.547	12.126	0.465	0.347	0.170	10.904	38.39	33.03
CM0502		9.293	1.405	9.396	0.314	0.414	0.084	7.860	37.26	37.67
CM0602		9.315	1.435	6.959	0.236	0.377	0.087	5.841	37.92	28.33
CM0701		13.992	2.603	12.886	0.564	0.444	0.150	10.960	35.32	32.53
CM0802		9.351	1.814	8.939	0.325	0.380	0.096	7.642	36.86	35.24
CM0902		18.668	3.408	14.391	0.497	0.504	0.232	12.467	38.05	29.33

Appendix 1.5. Individuals' apparent digestibilities of fatty acids and macronutrients for the three experimental diets.

		Apparent digestibility of fatty acids %										A-Dig.*	A-Dig.	A-Dig.
		C10:0	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	total	of Nitrogen	of Fat	of CHO
Refined Diet	TD0101	100.0%	99.7%	98.5%	92.7%	100.0%	84.3%	93.8%	92.9%	92.5%	93.0%	90.8%	95.3%	93.5%
	TD0201	100.0%	93.1%	99.7%	97.6%	100.0%	96.8%	96.5%	93.9%	96.5%	96.6%	90.7%	97.3%	92.9%
	TD0402	100.0%	99.7%	98.7%	93.9%	100.0%	85.2%	93.0%	92.9%	93.6%	93.2%	90.0%	95.6%	96.1%
	TD0502	100.0%	100.0%	98.6%	93.1%	96.2%	80.2%	92.9%	95.7%	88.7%	92.6%	88.8%	95.3%	94.9%
	TD0601	100.0%	97.6%	100.0%	93.9%	100.0%	90.8%	93.1%	88.9%	91.7%	93.3%	88.4%	94.9%	93.2%
	TD0701	100.0%	100.0%	98.9%	93.2%	100.0%	75.2%	94.5%	98.1%	94.3%	93.0%	92.6%	96.2%	95.4%
	TD0802	100.0%	98.6%	98.3%	95.1%	100.0%	93.0%	95.5%	88.3%	95.4%	94.9%	89.7%	96.2%	93.6%
	TD0902	99.3%	99.7%	99.3%	96.6%	100.0%	88.9%	92.5%	98.3%	94.2%	94.5%	88.8%	94.3%	95.8%
	TD1002	100.0%	100.0%	99.4%	96.4%	100.0%	82.6%	96.9%	98.6%	91.6%	95.5%	90.3%	96.2%	94.7%
Fruit&Veg. Diet	FV0102	91.8%	79.4%	76.8%	86.8%	100.0%	74.4%	95.7%	95.9%	95.9%	85.7%	79.9%	88.2%	96.0%
	FV0301	82.0%	77.1%	75.4%	85.6%	100.0%	89.5%	87.1%	77.6%	93.2%	82.6%	79.9%	81.7%	91.1%
	FV0401	81.6%	76.6%	76.5%	87.1%	100.0%	90.2%	90.1%	85.5%	94.3%	84.0%	78.2%	83.9%	93.6%
	FV0502	93.0%	85.8%	82.2%	89.3%	100.0%	82.1%	96.4%	97.7%	96.5%	89.4%	84.3%	89.6%	96.7%
	FV0602	92.2%	86.7%	85.4%	91.2%	100.0%	86.0%	96.1%	97.7%	97.5%	90.7%	85.6%	91.6%	97.4%
	FV0702	88.1%	81.8%	80.6%	88.7%	100.0%	92.1%	93.4%	94.1%	98.2%	88.0%	78.7%	88.2%	95.6%
	FV0802	85.6%	83.0%	81.9%	87.9%	100.0%	87.5%	93.2%	91.3%	97.8%	87.6%	79.2%	87.9%	95.6%
	FV0901	88.2%	79.5%	78.1%	87.2%	100.0%	77.1%	94.8%	96.5%	98.6%	85.9%	84.2%	86.5%	97.0%
	FV1001	83.8%	76.4%	75.7%	86.0%	100.0%	77.5%	91.2%	93.3%	98.8%	83.5%	82.3%	85.5%	96.2%
Cereal Diet	CM0102	94.6%	99.8%	100.0%	96.4%	100.0%	91.2%	97.1%	98.7%	99.3%	97.2%	80.3%	94.9%	92.1%
	CM0202	100.0%	99.8%	99.3%	96.8%	100.0%	92.1%	95.4%	97.2%	98.5%	96.7%	82.3%	93.4%	91.2%
	CM0301	100.0%	99.5%	100.0%	96.3%	100.0%	85.5%	95.9%	96.5%	96.3%	95.9%	86.7%	94.7%	90.3%
	CM0401	100.0%	100.0%	98.2%	96.5%	100.0%	87.5%	94.7%	98.0%	97.9%	96.1%	81.9%	96.9%	91.9%
	CM0502	100.0%	99.7%	100.0%	96.4%	100.0%	91.2%	96.6%	98.7%	98.6%	97.1%	84.9%	94.5%	91.9%
	CM0602	100.0%	98.3%	99.3%	96.9%	100.0%	96.2%	95.3%	95.7%	96.9%	96.6%	84.6%	96.3%	91.3%
	CM0701	95.3%	100.0%	98.8%	96.7%	100.0%	93.1%	96.3%	95.6%	98.1%	96.7%	81.4%	95.0%	90.4%
	CM0802	100.0%	100.0%	100.0%	95.8%	100.0%	91.2%	96.0%	94.5%	95.8%	96.1%	80.6%	95.2%	89.9%
	CM0902	94.1%	100.0%	98.6%	96.3%	100.0%	91.7%	96.1%	95.7%	97.6%	96.3%	81.7%	95.1%	90.8%

* A-Dig. represents apparent digestibility.

Appendix 1.6. Determined and calculated ME (kJ/g DM) for the three experimental diets.

		Determined			Calculated using factorial models				Calculated using empirical models				
		GE	DE	ME	ME _{Atwater}	ME _{FDA}	ME _{British}	ME _{Livesey}	ME _{Levy}	ME _{M&P}	ME _{M&J}	ME _{Southgate}	ME _{Livesey}
Refined Diet	TD0101	23.367	21.780	21.096	21.869	21.118	21.027	21.391	21.495	21.306	21.269	21.427	21.178
	TD0201	23.384	22.002	21.445	21.823	21.079	20.991	21.349	21.537	21.330	21.294	21.456	21.206
	TD0402	23.189	21.829	20.966	21.637	20.896	20.802	21.153	21.079	21.148	21.111	21.262	21.020
	TD0502	23.519	21.900	21.214	21.960	21.236	21.179	21.525	21.532	21.463	21.428	21.606	21.356
	TD0601	23.463	21.680	20.861	21.897	21.144	21.097	21.456	21.496	21.399	21.362	21.534	21.276
	TD0701	23.463	22.090	21.320	21.897	21.144	21.097	21.456	21.496	21.399	21.362	21.534	21.276
	TD0802	23.404	21.881	21.153	21.964	21.200	21.110	21.503	21.263	21.354	21.320	21.524	21.201
	TD0902	23.404	21.818	20.973	21.964	21.200	21.110	21.503	21.265	21.354	21.320	21.524	21.201
	TD1002	23.381	21.815	20.969	21.948	21.193	21.098	21.487	21.422	21.339	21.305	21.507	21.188
Fruit&Veg. Diet	FV0102	19.726	17.806	17.080	18.896	17.754	16.845	17.365	18.051	18.081	18.024	17.750	17.643
	FV0301	19.729	16.856	16.357	18.890	17.748	16.837	17.357	18.179	18.083	18.026	17.742	17.646
	FV0401	19.729	17.252	16.590	18.890	17.748	16.837	17.357	18.179	18.083	18.026	17.742	17.646
	FV0502	19.710	18.132	17.277	18.844	17.713	16.736	17.260	17.790	18.063	18.005	17.710	17.621
	FV0602	19.903	18.589	17.847	18.813	17.706	16.770	17.272	18.253	18.263	18.207	17.945	17.841
	FV0702	19.715	17.763	16.868	18.880	17.741	16.846	17.364	17.796	18.073	18.017	17.742	17.637
	FV0802	19.728	17.752	17.045	18.886	17.746	16.788	17.313	18.176	18.080	18.022	17.738	17.639
	FV0901	19.730	18.017	17.265	18.895	17.755	16.839	17.358	18.179	18.085	18.028	17.755	17.649
	FV1001	19.697	17.798	17.061	18.859	17.732	16.785	17.300	18.157	18.057	18.001	17.724	17.624
	Cereal Diet	CM0102	19.847	17.686	16.684	18.282	16.621	16.132	16.815	17.848	18.060	17.981	17.386
CM0202		19.877	17.661	16.789	18.295	16.612	16.118	16.813	17.864	18.081	18.002	17.395	17.463
CM0301		19.848	17.702	16.817	18.282	16.620	16.131	16.814	18.188	18.060	17.981	17.385	17.452
CM0401		19.782	17.763	16.939	18.237	16.614	16.153	16.820	18.035	18.011	17.933	17.346	17.415
CM0502		19.849	17.787	16.831	18.285	16.626	16.132	16.814	17.845	18.060	17.982	17.390	17.454
CM0602		19.810	17.834	17.112	18.240	16.589	16.046	16.726	17.803	18.020	17.942	17.348	17.414
CM0701		19.848	17.539	16.715	18.282	16.619	16.130	16.814	18.075	18.060	17.981	17.385	17.451
CM0802		19.848	17.451	16.559	18.282	16.618	16.129	16.814	17.850	18.060	17.981	17.385	17.451
CM0902		19.848	17.625	16.882	18.282	16.620	16.131	16.814	18.188	18.060	17.981	17.385	17.452

Appendix 2. Menus and intake levels for the three experimental diets.

Refined Diet Menu

Meal	Food items	<u>Intake level</u>				
		1.0	1.5	2.0	2.5	3.0
		<u>Amount consumed (g)</u>				
Breakfast (7-9am)	Tip Top Mighty White bread with iron	27	40.5	54	67.5	81
	KRAFT Peanut butter	11	16.5	22	27.5	33
	Craig's Butter	4.5	6.75	9	11.25	13.5
	Tararua fresh Homogenised milk	250	375	500	625	750
Lunch (12-2pm)	Tip Top Mighty White bread with iron	54	81	108	135	162
	Mainland Cheese, Colby	20	30	40	50	60
	Craig's Butter	4.5	6.75	9	11.25	13.5
	Foodtown Ham, shaved	10	15	20	25	30
Dinner (6-8pm)	Cadbury Moro bar	39	58.5	78	97.5	117
	Irvines Pie, cheese & mince	91	136.5	182	227.5	273
	Watties Peas, green, frozen	30	45	60	75	90
	Watties Corn, whole kernel, frozen	20	30	40	50	60
	Watties Baby carrots, frozen	20	30	40	50	60
	Arnott's Farmbake cookies, Butter Shortbread	24.5	36.75	49	61.25	73.5
Snack	Eta salted Peanuts, roasted	30	45	60	75	90

Cereal Diet Menu

Meal	Food items	Intake level		
		1.0	1.5	2.0
		Amount consumed (g)		
Breakfast (7-9am)	Countryfree wholemeal bread, toast	36	54	72
	Craig's Jam, strawberry	14	21	28
	Craig's Butter	4.5	6.75	9
	Sanitarium Bran cereal	30	45	60
	Bulk Premium Apricots, Woolworths	30	45	60
	Tararua Calci-Trim, fresh non-fat milk	150	225	300
	Pure Cane Sugar (sachet)	3.25	4.9	6.5
Lunch (12-2pm)	Countryfree wholemeal bread, sandwich	60	90	120
	Mainland Cheese, Edam, processed	22	33	44
	Craig's Butter	4.5	6.75	9
	Foodtown Ham, shaved	20	30	40
	Just Juice, Orange with apple base	250	375	500
	Arnott's Vita-Weat crispbread	12	18	24
Dinner (6-8pm)	Foodtown Bread roll, wholemeal	50	75	100
	Watties Peas, green, frozen, uncooked	50	75	100
	Watties Corn, whole kernel, frozen	40	60	80
	Watties Baby carrots, frozen	40	60	80
	Watties Cottage pie	160	240	320
	Kavli Crispy Garlic Crispbread	20	30	40
Snack	PAK'N SAVE Muffin, Bran & Apple	75	112.5	150

Fruit & Vegetable Diet Menu

Meal	Food items	Intake level		
		1.0	1.5	2.0
		Amount consumed (g)		
Breakfast (7-9am)	Sanitarium Weet-Bix	17.5	26.25	35
	Bulk Premium Apricots, Woolworths	40	60	80
	Bulk Sultanas, Woolworths	20	30	40
	Tararua Calc-Trim, fresh non-fat milk	120	180	240
Lunch (12-2pm)	Burgen bread, mixed fruit	80	120	160
	Mainland Cheese, Edam processed	22	33	44
	Sun Apricot bar	27.8	41.7	55.6
	Bulk Elliot Pitted Prunes, Woolworths	30	45	60
Dinner (6-8pm)	Watties Potato, fried, frozen	100	150	200
	Watties Peas, green, frozen, uncooked	40	60	80
	Watties Corn, whole kernel, frozen	30	45	60
	Watties Baby carrots, frozen	30	45	60
	Watties Cottage pie	140	210	280
	Watties Tomato sauce	15	15	15
Snack	Nice & Natural Apricot & Almond bar	40	60	80

*Tea, coffee, sugar and pepper were allowed up to 3 sachets per day for each subject in all 3-diet groups, water and salts were not restricted.

Appendix 3. Recruitment Advertisement

Free Food for 10 days! Believe it! 30 Volunteers needed

A Masters Student from the Institute of Food Nutrition & Human Health is conducting research on the accuracy of the nutritional data printed on food product packaging.

Thirty people are required for this 10 day research trial. Food will be provided to you free of charge.

During the trial your energy requirements and expenditure will be monitored to assess the nutritional value of your daily diet.

From the information gained we will be able to tell you how best to monitor your food intake to meet daily energy requirements.

For more information please contact:

Maggie Zou
Dr. J.H. Green
S.M. Rutherford



M.L.Zou@massey.ac.nz
J.H.Green@massey.ac.nz
S.M.Rutherford@massey.ac.nz

Institute of Food Nutrition & Human Health
Massey University
Private Bag 11-222
Palmerston North

This study is approved by the
Massey University's Human Ethics Committee

Appendix 4. Information Sheet

An assessment of the accuracy of existing energy conversion factors for calculating metabolizable energy (ME) in NZ foodstuffs

- INFORMATION SHEET -

The reasons for conducting this research

Health surveys have shown that people whose weight remains stable tend to live longer, and have less cardiovascular disease, than people who show large variations in weight. Your diet plays a critical role in maintaining good health and weight balance. In addition, it must contain sufficient energy and nutrients to meet your body's requirements. If an individual's diet contains more or less energy than needed over a long period, body weight will either increase or decrease. Either could present a health risk. For example, thin people may reduce their resistance to disease. On the other hand, being overweight is associated with many major illnesses such as heart disease, cancer and diabetes.

Currently, many people use the nutritional data printed on food products to monitor their daily energy intake. However, these data, especially the energy value, are based on the work of Atwater (1902) who conducted 50 dietary experiments on humans at the beginning of this century. Therefore, a study to assess the accuracy of the nutritional values for modern food products is essential.

Aims of this research

- To evaluate the accuracy of the energy value printed on food products in New Zealand.
- To investigate people's dietary habits and ascertain the nutritional value of their daily diet.

Who will be involved in this research?

Volunteers

Volunteers who are healthy, not receiving medication, and aged between 20 and 60 years old are required for this study.

Researchers

Maggie Zou
Brett Guthrie
Dr. J H Green
Mr. S M Rutherford
Prof. Paul Moughan



Institute of Food Nutrition & Human Health
Massey University

What will you do during this study?

This is a 14 day study involving 4 days of ascertaining your normal daily diet, and 10 days on a set trial diet to measure its true nutritional value.

1. Sign the Informed Consent Form

A detailed explanation of the general purpose of this study, all the procedures, and the expectations of the researchers will be given to you during your first visit. You will also be asked to sign an Informed Consent Form.

2. Measure your energy requirement

During your visit at the beginning of this study, your weight and height will be measured. Your medical history, nutritional history and daily activities will also be recorded. Then your daily energy expenditure and requirement will be derived from these measurements. Your daily food intake during the study period will be individualised based on these data.

3. Assess your normal daily diet

You will be given a diary in which to record your normal food intake and daily activities over a 4-day period. This will be used by us to assess the nutritional value of your diet. At the end of the study, you will be given the energy content in your diet, your daily energy requirements and recommendations for your diet if required.

4. Determine the metabolizable energy (ME) of the diet

During the 10-day trial period, you will be asked to eat the individualised diet supplied free by us. If you would like to, you can have lunch and dinner together with other participants. All the food will be pre-packaged, and can be taken home from the Food Kitchen, located in the Riddet Building, Massey University. You are asked not to consume any extra food, alcoholic or energy-containing beverages. Your weight will be monitored throughout the whole trial. We would also like you to record your daily activities.

From the 5th day onwards, you will be asked to collect your total faeces and urine for 6 days. All the necessary containers and bottles will be supplied. The detailed procedures for collection will be explained to you during the first visit. All the foods to be used in this study will be typical, common New Zealand foods. No gastrointestinal problems are expected from consuming these foods.

5. The Experiment Schedule

Day	Diet	Measurement
Visit	Usual diet	Interview – At Massey: <ul style="list-style-type: none"> • Sign consent form • Ascertain medical history • Recall your 24 hour food intake • Measure your energy requirement • Explain the experiment and expectations • Demonstrate sample collection procedures
Day 1 – Day 4	Usual diet	At home <ul style="list-style-type: none"> • Record your dietary intake for a 4 day period • Record your daily activities
Day 5 – Day 14	Trial Diet	<ul style="list-style-type: none"> • Consume the trial diet • Weigh yourself daily • Record your daily activities
Six day collection (Day 9 – Day 15)	Trial Diet	<ul style="list-style-type: none"> • Collect your total faeces • Collect your urine

Your rights, the risks & benefits of this study

You are most welcome to ask questions throughout the study. Any questions and concerns you may have during the study will be addressed as quickly as possible by the researchers.

Confidentiality will be preserved by giving you a code number that will subsequently be used on all forms, samples and data records. Identifying information will be stored separately from the logbook and data records. Your personal information will only be used for this research and publications arising from this research project. Your name will not be used. You may withdraw from the study at any time. We will reimburse the associated travel costs at \$2 per 10 km.

There are no direct risks to you in this study. From the information gained we will be able to make recommendations for your food intake in relation to your daily energy requirements.

You will be invited to attend the seminar where the results of the study will be presented by the researchers. At the end of the study you will receive an individual assessment of your diet including the energy content of your diet, your daily energy requirements and recommendations for your diet if required.

Appendix 5. Consent Form

An assessment of the accuracy of energy conversion factors for calculating Metabolism Energy value in New Zealand foodstuffs

CONSENT FORM

I have read the Information Sheet and have had the details of the study explained to me. My questions about the study have been answered to my satisfaction, and I understand that I may ask further questions at any time.

I also understand that I am free to withdraw from the study at any time with a written form to state my wish to withdraw, even after I have begun to take part. I am free to decline to answer any particular questions in the study. I agree to provide information to the researchers on the understanding that it is completely confidential.

I willingly agree to participate in this study under the conditions set out on the Information Sheet.

Participant

Signed: _____

Name: _____

Date: _____

In the presence of :

Researcher

Signed: _____

Name: _____

Date: _____

Appendix 6. Interview Documents

Personal Profile

All personal information listed on this sheet will be confidential, and this sheet will be destroyed after the trial is finished.

Name

Date of Birth

Gender

F / M

Work Location

Height(cm)

Weight(kg)

Before study:

Trial Diet

After study:

Fruit & Vegetable Diet

.

Estimated BMR (kJ)

Cereal Diet

.

Refined Diet

.

Activity Factor

Estimated Energy Expenditure (kJ)

General Comments

1. When are you available to start the trial?	
2. Are you able to bring your urine & faecal samples to the lab each day?	
3. Would you like to have lunch & dinner with other people recruited in the trial at Massey?	

4. What time would suit for you to collect your meals each day?	
5. Do you have a microwave oven at home?	
6. Do you prefer tea or coffee?	

Health

1. Are you currently in good health?	
2. How often do you have normal bowel movements?	
3. Do you have any restrictions on the type of foods you consume, e.g. food allergy, or religious belief?	
4. Have you ever had any surgery before? If so, please describe briefly.	
5. Do you currently take any food supplements, such as mineral tablets or vitamins?	
6. How often do you exercise? Briefly describe your daily activity schedule.	

Food Record Diary

Please record everything you eat and drink during the next four days. Instructions and an example are given inside. Information included here will be treated in confidence and results will be returned to you at the end of the study.

Name _____ Date of start recording ____/____/____
Address _____

Instruction for using the food diary

Everything that you eat and drink over the next four days should be recorded in the diary. The amount of food will be measured with a scale (if you have one) or household measures (such as cups, tablespoons), or estimated by portion (such as a quarter of 400g of pizza will be about 100g). For the snack foods, you can use the weight printed on the packet directly. Don't forget to record the food you eat away from home. Please remember to record separately the weight of tea, milk and sugar put into a drink. Please eat what you would normally eat. Don't change your diet for the recording period.

Names and descriptions of foods should be as detailed as possible, including the brand name and any other information available (such as recipes for food mixtures, preparation methods).

eg. Cheese – is insufficient information.

Cheese, cheddar (Shape reduced fat) – is sufficient information.

Start a new page in your diary for each day, and record each item on a separated line. Record the time of the day in the first column of each line.

eg. 10.30am Tiffany Mango cream biscuit 2 slices 20g

The space provided at the foot of each page for general comments is for you to give any further information about your diet and your activity for that day.

eg. Steady run, morning 1 hour.

Missing lunch due to stomach pains.

Please try to be as accurate as possible and try to choose a fairly typical day to record. For instance do not record a day when you are on holiday or when you are ill, if you feel that this would alter your normal diet or activities.

Appendix 7. Experimental Instruction

Accuracy of Energy Value Trial - Information for Participants -

Welcome into our trial! We greatly appreciate your taking part. Without your support, this study could not take place. Your conscientiousness and patience during the trial will determine the accuracy and precision of the results. Please read and follow the procedures outlined below. If you have any questions, please let us know. We will be very pleased to answer your questions and assist you at any time. Thank you for your time.

Our contact Ph. Number: Maggie Zou
Brett Guthrie



Food and Drink

- A whole day's meal will be pre-packed for you and stored in the Food Kitchen, Riddet Building, at Massey. You are asked to pick it up the day before it is to be consumed. If you are unable to come to Massey, please let us know and we will arrange delivery for you.
- There is a food list and cooking instructions in each package. Please check that all the foods listed are included in the package before you eat the meal.
- We prefer you to eat each meal at a regular, i.e. suggested, time. If you have to change meal times on a day, please make a note on the food list. Food is packed in each individual meal portion in the meal package. If you cannot finish all the food in one meal at the suggested time, you can eat them later. Please try not to swap meals with each other. Milk is provided for daily consumption. Snacks provided can be eaten at any time.
- Please only eat the food we supply and finish all. If the amount of food we have provided is too little or too much for you, please let us know. We have several intake levels for each diet chosen at the beginning of the trial.
- Please do not eat extra food, alcoholic or any energy containing drinks. It will affect the result. You can drink as much water as you like. *Diet* soft drink is allowed. Please record the amount of *Diet* soft drink you consume daily. We will let you choose tea or coffee for your daily drink at the beginning of the trial. A

maximum of three teabags or three coffee bags is allowed for a day. If you do not feel well during the trial or you have any questions about your diet, please feel free to contact us. Our contact phone numbers are listed above. We will discuss your concerns immediately. In some day if you really can't help yourself eating extra food, we would rather you tell us and make a note what you eat, full detailed information as possible, otherwise your result won't be accuracy. You are asked to eat all the foods we supply daily. In case you have any leftovers, please return them to us daily. We also ask you to return the empty packages of butter, jam, tomato sauce and juice, and make a note on how many bags of tea, coffee, sugar and salt you consume.

- We would like you to record your daily activities during the trial period to monitor your energy expenditure.
- The cooking instruction will be supplied for the foods that require cooking. Please follow the instructions. Care should be taken not to overcook any food.

Sample collection

- Faecal sample -

From day 5 of the trial diet onwards, we ask you to collect your total faeces for 6 days (7 days if you agree, then we will provide meals for one more day). Bowls and bags will be given to you for this purpose before the collection period starts. A chillibin and empty containers will also be available to you. Ice is available in Rm166, AgHort building. The collection procedures are listed below.

- Cover the bowl with the plastic bag labelled with your code, collecting date and trial day. Insert the bowl into the toilet before you empty your bowels.
- Please avoid contaminating your faecal sample with urine and blood. If contamination occurs, please make a note of it, and separate the sample as well as you can.
- Toilet paper should not be included in your collecting bag.
- After you have collected the sample, seal the bag with the rubber loops and put it in the container with the lid on. This container should be kept in the chillibin until it is transferred to the lab freezer.
- Please bring your sample to Massey every morning. If this is not possible, please leave your chillibin somewhere outside your house. We will collect samples twice each day.
- After collecting your sample, please wash your hands with the anti-bacterial soap that we will give to you at the beginning of the trial.

- 24 hour Urine collection -

- You are also asked to collect your urine from day 5 of the trial diet onwards. It is based on a 24 hour period.
- We suggest that women use same method as for the faecal sample collection, whereas men can use the bag directly. There is a 1.5ml eppendorf containing 0.8ml of 6M HCl in each bag. Hold the eppendorf through the bag, and open it before urination. Care should be taken not to spill the acid. If a spillage happens please rinse with plenty of fresh water. After your urine sample is collected you will seal the bag with a twist ties. Then the bag will be put in a container in the chillibin.
- All the bags will be labelled with the participant's code & collection time. We would like you to bring the sample to the lab each morning. We will collect samples twice a day if you are unable to bring them to Massey.
- If you forget to collect urine at some stage during the day, please make note of it, or send one empty bag labelled with that time to us.

Thank you for taking the time to read this. If you have any questions, you are most welcome to call us at any time. I hope you enjoy your meals.

Food Preparation Instruction

All the food except milk will be bought in bulk before the ten day trial period, with individual meal portions of each ingredient being weighed and packaged separately.

1. Bread, ham will be bought from Foodtown, weighed into individual portions ($\pm 1.0g$ of required weight), wrapped and frozen.
2. Dried apricots, prunes, sultanas, roasted peanuts and breakfast cereals will be bought from Toops and Woolworths in bulk. Each will be weighed into individual portions ($\pm 1.0g$ of required weight), packed into small containers and stored in the refrigerator.
3. Crispbread and shortbread will be bought from Foodtown, weighed and packed into small bags for each individual portion. These are stored in the Food Kitchen.
4. Frozen potato fries, peas, corn and carrots will be bought from Toops in bulk. These will be weighed ($\pm 1.0g$ of required weight) into individual meal portions and kept in the freezer until use.
5. Apricot bars, Apricot & Almond bars and Moro bar will be bought from Foodtown and Woolworths as a single pack size. They will be re-weighed before given to participants.
6. Mince'n'Cheese pies and Cottage Pies will be bought from Woolworths, weighed into individual meal portions, and kept in the freezer.
7. Fruit juice will be bought from Toops in 125ml and 250ml sizes.
8. Butter, peanut butter, jam, tomato sauce, sugar, salt, tea, coffee and pepper will be bought from Toops in sachet size. They will be re-weighed before given to participants.
9. Milk and cheese will be sponsored by Tararua branded milk, Longburn branch. Milk will be picked up freshly as required, then weighed ($\pm 1.0g$ of required weight), poured into bottles and stored in the chillier. Cheese will be weighed ($\pm 1.0g$ of required weight) and stored in the freezer.
10. Bran & Apple muffin will be bought from PAK'N Save, weighed into individual meal portions, and kept in the freezer.
11. The weight of each ingredient will be recorded carefully on the worksheet.

12. Each meal portion will be packed separately. A whole day meals will be packed on one food tray, wrapped with clear film ready for picking up.

All the meal portions will be labelled with the meal name, trial date, participant code and diet type. Different colour Sellotape will be used to identify the type of diet.

Food preparation will be conducted in the Food Kitchen, Riddet building. Before pre-packaging the food, all the bench and facilities will be cleaned hygienically. People working in the Food Kitchen must wear suitable protective clothing including a hat and gloves. During the preparation, a face mask will also be used. Cooked and uncooked food must be treated separately. All the containers used for packing must meet food hygiene standards. No food will be touched with bare hands.

Food will be picked up or delivered from the Food Kitchen daily, i.e. the day before being required. Before a food package leaves the Food Kitchen, it must be double-checked with the code label, food content and trial date.

Frozen Vegetables, potato fries and pies will be cooked by participants at home. Cooking instructions for food requiring cooking before serving will be included in each meal package. Care should be taken not to overcook any food.

1. The container with mixed vegetables can be put into a microwave, cooked on High for 3 minutes stirring only once (Based on 700 watt microwave). If a participant does not have a microwave at home, they can boil the vegetable instead of microwaving. Place one cup of water into a saucepan to boil, then add mixed vegetables into the boiling water, return to boil and continue to cook for 3 minutes.
2. Potato fries will be cooked in the oven. Preheat oven to 230°C (210°C for fan forced oven). Spread fries in a SINGLE LAYER on an oven tray. Cook for 16 to 20 minutes (conventional oven) or 12 to 14 minutes (fan oven).
3. Cottage Pies will be heated either in a microwave or in a conventional oven.
 - In a microwave: Remove pie from cardboard pack, pierce overwrap.

Place wrapped pie in microwave.

Microwave on HIGH for 6 minutes (for 650–700 watt microwaves) or until thoroughly heated.

Allow it to stand for 1–2 minutes.

Carefully remove from oven and remove protective overwrap before serving.

- In a conventional oven: Preheat oven to 180°C (350°F).

Remove pie from cardboard pack. Remove the protective overwrap.

Place frozen pie on an oven tray in the centre of the oven.

Heat for 30-35 minutes.

Carefully remove pie from oven on the tray

NB: Dish may melt if placed too close to heating element.

All the participants will be asked to only eat the food we supply. Tea or coffee can be chosen by participants at the beginning of the trial. Each participant will be allowed a maximum of two tea/coffee bags each day. Three salt bags and two sugar bags will be given to participants each day. Participant can drink as much water as they like. Diet soft drink is allowed during the trial, but participant will be asked to record the amount they consume each day. If some participants can't help eating extra food during the trial, they will be asked to record the detail information on a daily basis.

Each diet (a whole day's portion) will be collected on five occasions (i.e. day 1, day 3, day 5, day 7 and day 9) in total. On each day, the food will be treated (such as cooked in the oven) in the same way as the participant's diet, then it will be poured, frozen for further analysis. In addition, each of ingredients will be collected at the same occasion as the participants' meals and kept in the freezer until freeze-dried.



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**FACULTY OF
BUSINESS STUDIES**

DEPARTMENT OF
HUMAN
RESOURCE
MANAGEMENT

17 July 1998

**Maggie Zou
Institute of Food Nutrition and Human Health
MASSEY UNIVERSITY**

Dear Maggie

Re: Human Ethics Application HEC 98/123

*An assessment of the accuracy of existing energy conversion factors for
calculating metabolizable energy (ME) in NZ foodstuffs*

Thank you for your amended information sheet and protocols.

The amendments you have made now meet the requirements of the Human
Ethics Committee and the ethics of your proposal are approved.

Yours sincerely

**Professor Philip Dewe
Chairperson
Human Ethics Committee**