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 University, Palmerston North, New Zealand

MAGGIE LONG ZOU

2007

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 (±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.

ACKNOWLEDGEMENT

I have been very fortunate to be able to conduct my Master of Science degree under Professor Paul J Moughan's supervision. I wish to express my sincere appreciation to Professor Paul J Moughan, for his unconditional commitment, guidance, encouragement and patience throughout the study. My gratitude is also expressed to Dr Ajay Awati for his valuable advice and priceless support during the final stage of thesis preparation.

I would like to thank my lab manager, Ms Fliss Jackson, for her valued support and friendship over the years. My thanks also go to my employer, Massey University, for giving me the opportunity to be able to pursue my studies while working.

Special thanks go to all the participants in my trials, for their willingness, enthusiasm and commitment. Without their loyal and devoted cooperation, completion of this study would be impossible. I also acknowledge the Mainland Products Ltd, Longburn site, New Zealand, for their support by providing all the dairy ingredients required for this study.

There are many people I wish to thank for their friendship, assistance and support during the course of this study. In particular I would like to thank Dr Hilary Green and Mr Shane Rutherfurd for their advice in developing experimental protocols and in preparing documents needed for recruitment of participants and obtaining ethics approval to conduct the human trial. Thanks to Mr Colin Leong, Mr David Luo, Mr Brett Guthrie, Mr William Su and Mrs Lyn Wang, for giving so generously of their time to assist with the trial work. I am also grateful to Mr Shane Rutherfurd and Dr Kay Rutherfurd-Markwick for their valuable advice and assistance in editing the draft of the thesis, especially to Mr Shane Rutherfurd who was always my first port of call for help. Thanks to Dr Patrick Morel for assisting me with statistical analysis; and also to A/Professor Hugh Morton and Dr Jonathon Godfrey for explaining the theory behind the data analysis.

My heartfelt appreciation is extended to my family and close friends for their endless encouragement and support, which helped me get through the highs and lows of my life as a Masters student.

Finally I would like to dedicate this thesis to my father, who always believed in me, and watches and protects me from heaven.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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 Burkit 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
			Resistant starch granules	Raw potato and banana	Resistant
			Retrograded amylose	Cooked potato after cooling	Resistant
	Non-starch polysaccharides	Cellulose		Plant cell walls	Largely resistant
		Non-Cellulosic polysaccharides	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).

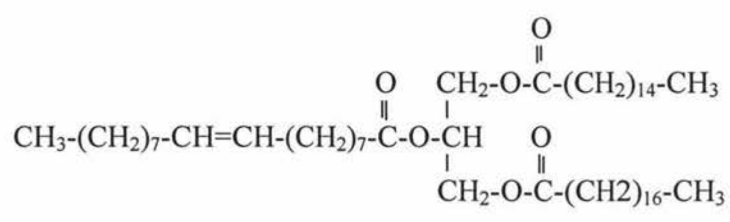

Figure 1.1. Structural formula of a typical triglyceride

Over 40 different fatty acids (FA) are found in nature, they have a basic formula $CH_3(CH_2)_nCOOH$ 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	Tetranoic 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**.

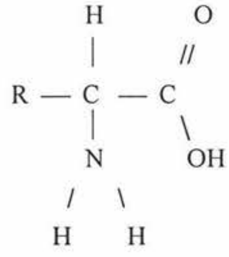

Figure 1.2. The general formula of an amino acid. R represents the side chain, which determines the specific characteristics of each individual amino acid.

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 food[1].

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 conductive 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).