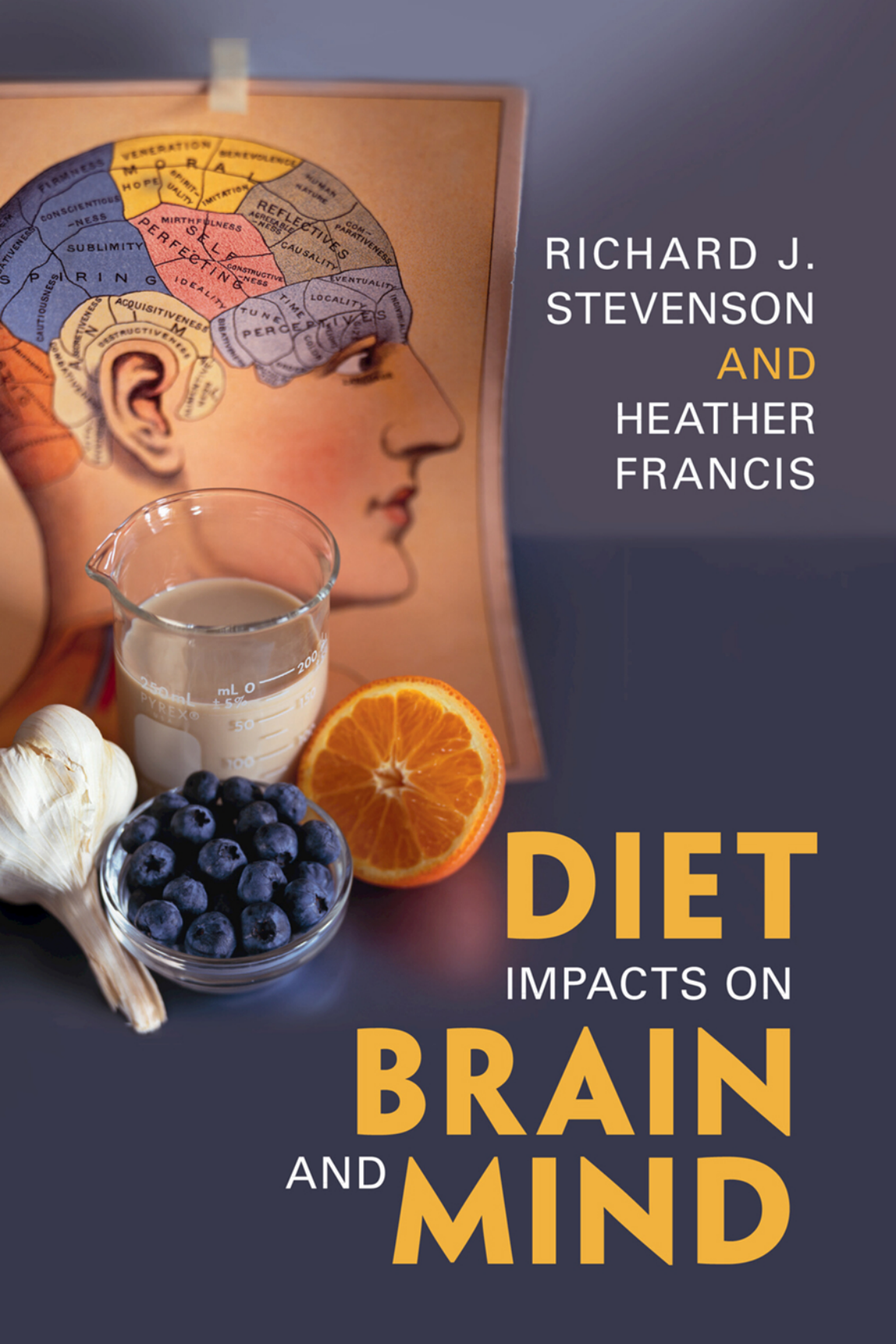

RICHARD J. STEVENSON AND HEATHER FRANCIS

DIET IMPACTS ON BRAIN AND MIND

DIET IMPACTS ON BRAIN AND MIND

Everybody eats, and what we eat – or do not – affects the brain and mind. There is significant general, applied, academic and industry interest about nutrition and the brain, and yet there is much misinformation and no single reliable guide. *Diet Impacts on Brain and Mind* provides a comprehensive account of this emerging multidisciplinary science, exploring the acute and chronic impacts of human diet on the brain and mind. It has a primarily human focus and is broad in scope, covering wide-ranging topics like brain development, whole diets, specific nutrients, research methodology and food as a drug. It is written in an accessible format and is of interest to undergraduate and graduate students studying nutritional neuroscience and related disciplines, healthcare professionals with an applied interest, industry researchers seeking topic overviews and interested general readers.

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DIET IMPACTS ON BRAIN AND MIND

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Preface and Acknowledgements

This book was born of a simple need. When we first started studying Western-style diets and their impacts, we wanted a general introduction to the broader field of diet, brain and mind. We could find no single source that gave us the overview we desired, and so we decided to write one. Writing a book is always a collaborative endeavour, and many people have helped us in this process. We thank Margaret Allman-Farinelli, Jen Cornish and Deb Mitchison for kindly reading and commenting on some of the chapters; Bob Boakes, Jon Mond, Tuki Attuquayefio, Martin Yeomans and Terry Davidson for many enlightening discussions; and Alysia Robertson, Karina Chan and Fiona Wylie for assistance with the References. We thank the Australian Research Council for their continued support, which assisted with much of our research reported in this book. Dick Stevenson particularly thanks his family for their support – Caroline, Gemma, Lucy, Harry, Chris, Mike, Rosie and Bailey – through good times and bad, and his dear ex-neighbours Charles Kamerman and Jennifer Cheyne for letting him help with Charles's book (in a very small way), which inspired him to work on this one. Heather M. Francis thanks her many supportive colleagues and friends who have provided guidance and wisdom – especially Dick Stevenson, without whom she would never have written this book. She would also like to give a special thanks to her family – Audrey, Gemma and Chris – for being an endless source of joy and inspiration. Finally, both of us thank Stephen Acerra of Cambridge University Press for his unerring support throughout the life of this project.

CHAPTER 1

Introduction

1.1 Introduction

The aim of this book is to provide a comprehensive account of the acute and chronic impacts of human diet on the brain and mind. Importantly, this is distinct from the much larger literature studying how the brain and mind affect food intake. It is distinct because in this book, the presumed causal arrow generally points from food → to brain and mind. A further aspect of this book is its emphasis on humans. This is both pragmatic (e.g., for health and policy implications) and reflects our interests in understanding the effects of diet on the human brain and mind. While there is a primary human focus, we have by necessity drawn on the animal literature. Unlike human studies, animal research can get nearly 100% compliance with experimental dietary regimens, and it is possible to undertake studies that are difficult to do with people, especially those concerning mechanism. Inclusion of animal data is also based on the premise that humans and animals share much common biology. In Sir Austin Bradford Hill's consideration of how to establish causality (Hill, 1965), scientific plausibility (i.e., is there a mechanism?) and coherence with known facts were two key criteria. Animal data is very important as they are particularly useful for understanding mechanism (i.e., scientific plausibility), and for experimental demonstrations of dietary effects on brain and mind (i.e., coherence).

As the title implies, the book investigates the impacts of diet on brain *and* mind. It seems important to study both, although the relative emphasis shifts between chapters dependent on what is known and the topic. It is essential to study effects on mind (operationalised as behaviour and cognition) because this level of explanation has great practical utility. If breakfast makes children concentrate better, intermittent hunger makes people immoral, and fruit and vegetables make people happy, it is important to know this *irrespective* of how diet causes these effects in the brain. Notwithstanding, it is also important to determine how diet does these

things to the brain. This establishes mechanism, with its implications for the biological plausibility of any observed effect on mind. It can also provide information for human betterment, via say developing nutraceuticals, drugs or other forms of treatment that target the neural mechanism. That aside, the pursuit of knowledge for its own sake is a worthy goal, and it was in this spirit that our book was conceived and written.

There have been many excellent and pioneering books on diet, brain and mind, all of which have appeared as edited volumes (e.g., Lieberman, Kanarek, Nehlig, Dye, Watson). However, these can sometimes lack a consistent approach between chapters, with, for example, greater or lesser emphasis on animal data, epidemiology over experimental work, or whatever. Coverage of the field is also sometimes limited to particular parts, reflecting the interests and expertise of those authors and editors. To date, nobody has tried to pull all of the different strands that make up the field of diet, brain and mind into one volume. Nor has there been a consistent focus on humans, with an interest in both brain and mind. As we have discovered, the field is much larger than we originally thought, it is also very diverse and its parts are often disconnected – but it is endlessly fascinating. The field also faces some significant methodological challenges, both in accurately measuring and manipulating human diet and in measuring brain and behaviour. However, scientists are an ingenious bunch and they have risen to the challenge.

The book proper starts with Chapter 2 on pregnancy, breastfeeding and infancy, followed in Chapter 3 by the acute effects of food intake, looking both at specific meals (e.g., breakfast) and specific nutrients (e.g., particular amino acids). Chapter 4 examines the chronic effects of food intake, with special emphasis on the major dietary pattern found in developed, and now developing, countries: a Western-style diet, rich in saturated fat, salt and added sugar. Chapter 5 explores the acute and chronic effects of dietary neurotoxins, coming both from foods and their contaminants (e.g., fungi, pesticides). Diet can also have an important protective effect on brain and mind, and indeed this is increasingly being recognised as a potential intervention for psychiatric, neurological and neurodegenerative conditions. This is all examined in Chapter 6. In addition, both Chapters 4 and 6 include emerging data on how diet affects the microbial ecology of our large intestine, as these organisms may have an important role in how diet impacts brain and mind. The food and drink we consume are the major routes for ingesting two of the world's most popular drugs, alcohol and caffeine. It has also been suggested that certain foods – ultra-processed items that bear little resemblance to the ingredients from which they are

made – may exert drug-like effects (e.g., dependence, craving) in consumers. Drugs and food form the basis for Chapter 7. Chapter 8 examines the science of starvation, both its acute and chronic effects in and outside of the laboratory, and the use of energy-restrictive diets for life extension. Chapter 9 looks at the impact on brain and mind of specific nutrient deficiencies (i.e., vitamins, minerals and certain essential macronutrients). Finally, Chapter 10 provides a reflection on this content, its implications and where the field might (and perhaps should) be heading.

The remainder of this first chapter has two aims. The first is to provide a brief overview of the core knowledge and methods that underpin research into diet, brain and mind, and their limitations. We have included this because readers coming afresh to this area may have experience in one domain (e.g., nutrition) but not another (e.g., psychology, brain science), or indeed no experience at all. The second concerns our focus. We have already identified that the emphasis is on humans, and brain and mind, but there is another aspect to our approach that is best discussed with some understanding of the strengths and weakness of the available methods, as they relate to measuring diet. Hence, we have left a discussion of this topic until the end of the chapter, assuming that those unfamiliar with the nutrition literature will first read the following relevant parts first.

1.2 Basic Nutritional Concepts

The main purpose of eating is to satisfy the body's energy needs (Woods & Seeley, 2000). In addition, eating provides the materials for growth and/or maintenance of all bodily systems. In humans, eating and drinking are also a major source of pleasure – if not an art as gastronomy – and a major vehicle for intoxication (e.g., alcohol, caffeine). These more uniquely human aspects of ingestion are of great social and scientific interest. This is because they contribute in no small part to: (1) overeating and obesity; (2) drug abuse, with alcohol being one of the most frequently misused; and (3) to the abuse of other substances which hijack multiple aspects of the brain's appetite/reward systems that support feeding.

Humans obtain energy from four main constituents of what they eat and drink – carbohydrates, proteins, fats and alcohol (Eschleman, 1996). Setting aside alcohol, the three main energy-yielding constituents of food are termed macronutrients. The amount of energy in a food or a drink is measured either in the SI unit the joule (and typically in kilojoules (kJ)) – which is used in this book – or alternatively, and mainly in the United States, by the calorie (and again typically as the kilocalorie (kcal); to

convert, 1 kcal = 4.18 kJ, and 1 kJ = 0.24 kcal). The formal definition of the calorie is a lot easier to grasp than the formal definitions of the joule. The calorie is defined as the energy required to heat a gram of water by one degree centigrade. The joule is easier to define informally as the energy required to lift a large tomato one metre into the air (or if you prefer more formally – the amount of work done when a force of one Newton is used to move an object one metre in the direction of the applied force (i.e., 1 N·m)).

The energy needs of an individual vary markedly, dependent on age, body composition (muscle vs fat), body weight, pregnancy, lactation, health, activity and climate (Eschleman, 1996). A person's basic energy requirement is the amount of food in kJ they need to eat to maintain their basal metabolic rate. Basal metabolic rate reflects the essential operations of the body necessary to maintain life at rest (i.e., cellular metabolism and maintenance). A man requires around 4.2 kJ per kilogram per hour to maintain basal metabolic rate and a woman around 3.8 kJ. Thus an average US man has a daily energy requirement just to meet basal metabolic rate of around 8,000 kJ and a woman needs around 7,000 kJ. Basal metabolic rates vary markedly over the lifespan, and hence so do energy needs. An infant requires 9.3 kJ per kg per hour, while an elderly woman needs 3.4 kJ per kg per hour. Illness can dramatically increase basal metabolic rate. A change in body temperature from 37 to 41 degrees centigrade due to a fever requires an approximately 60% increase in basal metabolic rate. This is one reason why infection has more lethal effects among starving people.

The other important component in determining energy needs is to establish that spent on moving around and doing things. As activity levels are generally quite low in the developed world, multiplying the adult basal metabolic rate requirement by 1.3 gives a rough guide to typical ideal adult energy intakes (i.e., 10,400 kJ for a man and 9,100 kJ for a woman). For a highly active adult (e.g., a lumberjack), doubling the basal metabolic rate requirement is necessary to satisfy total energy needs.

All of the three macronutrients (and alcohol) can be metabolised to provide energy for the body, and surplus energy from all three sources (and alcohol) can be stored as fat (Eschleman, 1996). Carbohydrates and proteins yield around 17 kJ per gram, while fats provide 38 kJ per gram. Under normal circumstances, carbohydrates provide the main energy source for humans. In our ancestral environment, complex carbohydrates were the principal energy source in the form of starch (e.g., tubers (potatoes, cassava), grass seeds (wheat, rice)), with indigestible complex

carbohydrates providing fibre (cellulose, inulin). Starch is composed of multiple glucoses units connected by covalent bonds. This is broken down in the digestive system into glucose. Glucose is a monosaccharide, and is used by the body as an energy source. It is present in blood at around 1,400 mg per litre in a healthy adult, and is stored in small amounts in various bodily depots as glycogen. Glycogen is a polysaccharide, with chains of glucose attached to a glycogenin protein core. Depots of glycogen are found in muscles (about 500 g in total) and the liver (about 100 g). Maintaining adequate supplies of glucose is essential because it is the primary energy source for the brain, where the *only* alternative fuel is ketone bodies (essentially an emergency fuel when all potential sources of glucose are exhausted). Nerve cells are unable to store glucose as glycogen.

In contrast to ancestral diets, a significant source of carbohydrates in modern diets comes from one particular disaccharide – sucrose or sugar. Disaccharides are composed of two monosaccharide units. Glucose is a monosaccharide, and there are two other important monosaccharides. Fructose, which is particularly sweet, and galactose, which is not that sweet – with both of these found in small quantities in certain fruits. The most important dietary sugar is the disaccharide sucrose, which is made of one glucose unit and one fructose unit. In the United States, each person consumes an average of 20 teaspoons (80 g) of sucrose per day (Drewnowski & Rehm, 2014). Other important dietary disaccharides are lactose ('milk' sugar), found in mammalian milk (composed of a glucose and a galactose unit), and maltose, found in beer (composed of two glucose units). Neither lactose nor maltose are particularly sweet.

As noted earlier, glucose is the principal fuel for the brain as well as being a major bodily fuel (Sembulingam & Sembulingam, 2016). In the presence of oxygen (i.e., aerobically) it is converted into pyruvate-liberating energy, and further energy can be released by the conversion of pyruvate into acetyl coenzyme A. All of this takes place in the cytosol (i.e., in the main portion of the cell). Acetyl coenzyme A is then fed into the Krebs cycle, which takes place inside mitochondria (a cellular organelle), liberating yet more energy (see Figure 1.1). Under conditions of high exertion, when the body cannot supply sufficient oxygen to muscle tissues for aerobic respiration (i.e., energy generation), both glucose and pyruvate can be metabolised without oxygen (i.e., anaerobically) in the cytosol, providing a brief burst of energy, but leading to a rapid build-up of lactic acid, which inhibits further anaerobic respiration.

In the absence of adequate supplies of carbohydrate, protein can serve as a good fuel substitute, as approximately half of the protein available in diet

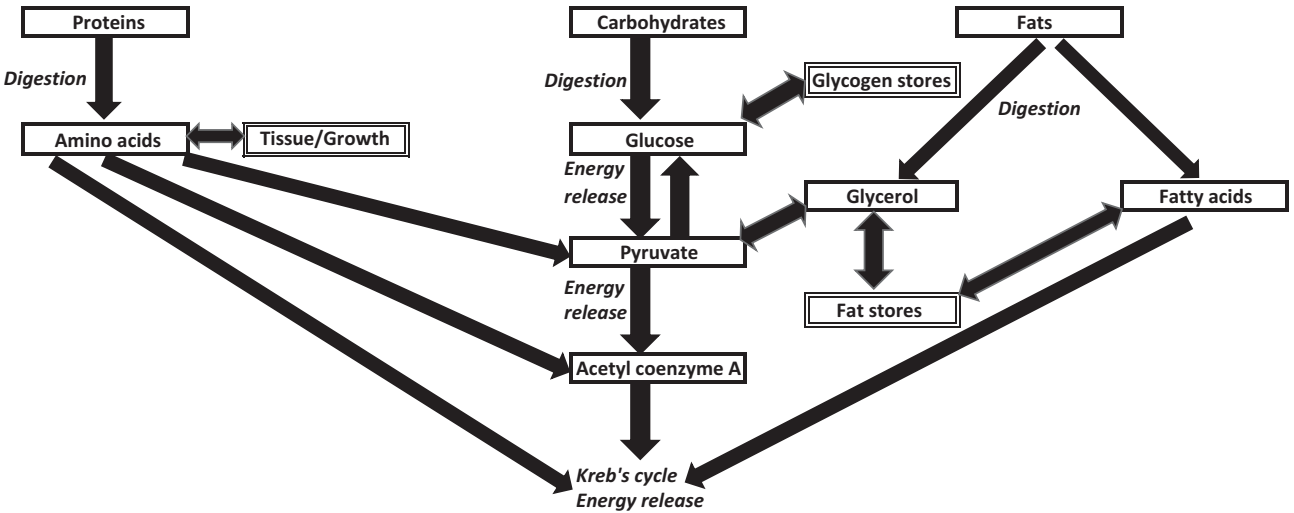

Figure 1.1 Basics of energy metabolism

can be metabolised to pyruvate, which can then be converted back to glucose (Sembulingam & Sembulingam, 2016). The remaining half can be fed directly into the Krebs cycle, the major energy-generation pathway for the body (see Figure 1.1). In the main, dietary protein, which is built from multiple, different amino acids, is essential for tissue maintenance and growth (i.e., creation of new proteins (e.g., enzymes) and biomolecules – like neurotransmitters), and for fluid balance. Humans require 20 different amino acids, some of which have to come from dietary sources as they cannot be synthesised by the body. Meat provides all of the required amino acids. A solely plant-based diet can as well, but care is needed to avoid insufficiency.

While fats are a good source of energy (see Figure 1.1), they are a poor source of glucose and are not used as a fuel by the brain. Fats are composed of a glycerol molecule, which can be converted to glucose, with three fatty acid tails attached, which cannot be converted (Sembulingam & Sembulingam, 2016). The type of fatty acid attached to the glycerol molecule dictates the type of fat – saturated, monounsaturated, polyunsaturated and trans-saturated. Type is based upon the presence/absence and location of carbon double bonds, and subtype by the length of the fatty acid chain. Different types of fats tend to be found in different types of food. Saturated fats, which are solid at room temperature, are generally from animals (e.g., meat, dairy), with the exception of palm and coconut oil. All of these are associated with an unhealthy blood lipid profile and have been linked to coronary arteriosclerosis and heart disease, although this is no longer a universal conclusion (Chowdhury et al., 2014). Monounsaturated fats have plant-based sources (e.g., olive oil, canola oil), and are linked to a beneficial blood lipid profile. With polyunsaturated fats, which are liquid at room temperature (as with monounsaturated fats), one common type is found in nuts and seafood (omega 3) and another in plants (omega 6). Both are linked to beneficial blood lipid profiles. Trans-saturated fats are factory-made from plant-based unsaturated sources. They have very useful properties in that they do not readily oxidise (i.e., go rancid) giving foods made from them a long shelf life. Unfortunately, they seem to have a worse effect on blood lipid profile than saturated fats and they have been banned in several countries. While fats are a major source of energy, they have many other important functions. They form key parts of cell membranes, and nerve fibre myelin sheaths, and they are needed for hormone synthesis, the digestion of certain vitamins, thermal insulation (subcutaneous fat), energy storage and organ padding.

In addition to the macronutrients, the body needs a range of micronutrients, all of which are provided in a typical omnivorous diet (Eschleman, 1996). Micronutrients are divided into vitamins and minerals. Vitamins are a heterogenous collection of organic compounds that the body cannot synthesise, which are necessary for normal function, and so have to come from food. Vitamins are usually grouped into those that are fat-soluble (A, D, E and K) and those that are water soluble (B, C). Dietary insufficiency in particular vitamins is linked to specific deficiency diseases, many of which have a long and tragic history (e.g., beriberi, scurvy, pellagra). In the past, these diseases were not recognised as resulting from a dietary cause (e.g., scurvy was thought to be a manifestation of syphilis). Identifying the cause required use of a scientific method. This was applied to scurvy by English naval physician James Lind in 1797. It revealed scurvy's dietary basis and probably represents the first ever clinical trial (Carpenter, 2003).

Mineral elements (beyond carbon, hydrogen, nitrogen and oxygen) are the other necessary dietary components. These are classified as either macrominerals (requirements greater than 100 mg per day) – calcium, phosphorous, sodium, sulphur, chlorine, potassium and magnesium – or microminerals (<100 mg per day) – iron, iodine, fluorine, selenium, zinc and several others. Deficiencies in these microminerals can produce severe disease (e.g., iodine deficiency, goitre and cretinism) and in some instances either too much or too little can be harmful (e.g., sodium/chlorine (salt); selenium).

1.3 Basics of Digestion and Regulation

The preparation for digestion starts before eating, as a variety of processes are triggered by the thought, sight or smell of food (think Ivan Pavlov, bells, and salivating dogs – the cephalic phase response). Food in the mouth is mechanically broken up by the action of chewing. It is mixed with saliva and formed into a bolus for swallowing (Longenbaker, 2017). Saliva contains the enzyme alpha amylase, which acts rapidly to break down starch into sugars. Whether this is to aid digestion or to promote consumption by making a starchy food taste somewhat sweet is not known.

The swallowed bolus passes down the oesophagus into the upper part of the stomach where it is ground into even small particles by this organ's muscular action. This ground food is then moved into the body of the stomach where it is mixed with acid and enzymes, forming a semi-liquid

called chyme which collects in the lower part of the stomach. The chyme is then expelled via the pyloric sphincter into the small intestine, which is the main organ for the absorption and digestion of nutrients in the human body.

The small intestine is a muscular tube 2–3 cm in diameter and 6–7 m in length (but probably less, as these measurements are based on cadavers with 'relaxed' muscles; Longenbaker, 2017). The small intestine has waves of muscular action (peristalsis) so as to move its contents progressively along its length. Because the wall of the small intestine is heavily invaginated and covered with a myriad small projections of tissue (microvilli), it has a large surface area, around 60 m^2 in an adult (a quarter of a tennis court). This assists effective nutrient absorption. The arrival of food into the small intestine triggers the release of cholecystokinin (CCK). This hormone stimulates the production of bile to break down fat, and the release of pancreatic juices to break down protein and carbohydrates. CCK also acts to slow the release of chyme from the stomach. Amino acids, glucose, fatty acids, vitamins and minerals then pass (some actively (i.e., energy driven transporters) and some passively) through the endothelial lining of the gut into cells. In these cells fatty acids are packaged into chylomicrons, which, together with other nutrients, are released into the hepatic portal vein for transport to the liver and other bodily tissues.

The remaining unabsorbed material passes into the large intestine, which is 1–2 m in length, and called 'large' due to its 6–7 cm diameter (Longenbaker, 2017). While digestively of lesser importance than the small intestine, it nonetheless has several functions. The large intestine is home to a vast number of microorganisms, which feed on the undigested produce coming from the small intestine. This microbial ecosystem is increasingly being recognised for its impact on health, as the type of organisms present influences the production of ketones, which serve as both an energy source, but also act to preserve the integrity of the endothelial (i.e., gut–body) barrier (Berding et al., 2021). Leakage of material from the gut, other than nutrients, may initiate a number of disease processes, possibly including dementia, as amyloid beta is produced in abundance by certain types of bacteria, but not by others. Many other factors also influence the integrity of the gut–body barrier, in particular the type of bacteria present. Diet can result in fairly rapid changes in this microbial ecosystem, for the better (plant-based foods) or worse (processed foods). Fermentation by gut microbes is also important in the production of certain B vitamins, and for vitamin K, and these alongside water and ketones are absorbed by this part of the digestive tract. Compactification

occurs, followed by temporary storage in the rectum, before defecation ends the digestive process.

The digestive system and the brain are connected in several ways. The brain's sensory systems 'see', 'smell', 'taste' and 'feel' food as it is eaten, providing information to prepare the organism for feeding and to terminate a feeding bout. The vagus nerve links the brain and gut, carrying sensory information (e.g., nutrient sensing, gut fullness) from gut to brain (most traffic) and some signals from brain to gut. Hormonal signals released by digestion, the digestive system and fat stores all impact brain function and regulation (Lowell, 2019). These include grehlin released by the stomach when empty, CCK when the stomach is filling or full, and leptin from fat, indicating the extent of bodily fat stores. All of these hormones affect appetite and exert influences well beyond this domain (e.g., grehlin promotes learning – a good idea if one is to remember a food source). The brain also monitors a range of physiological parameters which provide information about the nutritional status of the body, such as signals of muscle usage and blood glucose, for example. This information feeds into a number of brain areas that are known to be involved in the regulation of appetite (Logue, 2015). The most well-known component is the hypothalamus, particularly the lateral and ventromedial parts, which are early processors of sensory, gut, hormonal and metabolic signals and set up a general brain state either favourable or not for eating. The striatum, frontal cortex, orbitofrontal cortex, insula and hippocampus interact to regulate eating based on both hypothalamic outputs and in many cases via the same sensory, gut, hormonal and metabolic signals as well. These latter structures are the probable basis for the conscious aspects of eating, underpinning sensation, pleasure, feelings of fullness and hunger, thoughts about food, and broader conceptual considerations such as dieting, morality of meat eating or gourmet dining, for example.

While the brain is a major controller of food intake, certain regulatory aspects of digestive/ingestive processes are more peripheral. Glucose and fat are two important examples, reflecting the operation of short-term and long-term energy management systems, respectively. Increases in glucose, typically after eating, are kept in check by the release of insulin from beta cells in the pancreas, which sequesters excess glucose into muscle or liver cells as glycogen. In contrast, falling blood glucose leads to the secretion of glucagon by alpha cells in the pancreas, which liberates glucose from glycogen. For fat stores, which are the body's energy reserve against starvation, fat cells continuously release a hormonal signal leptin, which reflects bodily fat content. As leptin levels fall, indicating a reduction in

bodily fat stores, there is a significant stimulatory effect on appetite via the brain, while elevated leptin levels retard appetite. Impairments in sensitivity to the effects of insulin occur in type II diabetes, and insensitivity to the appetite retarding effects of leptin often occur with obesity.

1.4 Measurement

1.4.1 Diet

In the context of this book there are two dietary measurement issues that need to be addressed. The first relates to observing what a person has been eating. The second relates to experimentally manipulating what a person eats. The first is sometimes met with slow headshaking, a sharp intake of breath and warnings about the unreliable nature of dietary self-report data. The reality is not so bleak. It is possible to obtain *fairly* accurate measurements of what a person eats and there are three general methods that have been used to do this.

The most commonly adopted approach involves self-report (Thompson & Byers, 1994). The first form of this appeared in the 1930s, to collect diet information outside of a consultation with a dietician. Participants were asked to keep a diet diary for between 1 and 7 days. The approach is largely unchanged. The diary may be paper or electronic and is used to record everything that is eaten, in terms of when, what it was, and how much of it was consumed (the latter can involve weighing or visually judging portion sizes). Weighing each thing eaten and judging portion size are difficult for participants to do and are prone to a number of inaccuracies (Schoeller, 1995; Smith, 1993). Diary approaches have good short-term reliability, although this varies depending on the specificity of the dietary variable (i.e., being higher for global measures like overall energy intake – correlations of around 0.5–0.8) and they provide good detail about foods eaten. This method's weaknesses are compliance, as it can be burdensome to complete and the high cost of coding (i.e., each diary has to be converted into amounts of food, and then the nutritional value calculated – assuming it is available for that food).

A widely used variant of the diary approach is to interview participants about their previous day's food intake (24-hour recall), typically using a structured approach with probes (e.g., did you have a snack in the morning?). This method is widely used in nutrition research but has an additional *disadvantage* over the diet diary. As the interviewer is a real live human being, the interviewee, who is describing their diet, is likely to err

towards reporting foods and behaviours that they think will cast them in the most favourable light (Grimm, 2010). These types of demand effects are often unconscious and affect participant reports of any behaviour which may have negative personal connotations (e.g., unhealthy food, excessive drinking, certain sexual practices, etc.). While demand effects impact all forms of self-report, the presence of an interviewer probably accentuates this problem. This can lead to both under-reporting of nutritionally poor-quality foods (chips, chocolate, etc.) and their quantity consumed and over-reporting of healthy foods. The 24-hour recall method is also time and labour intensive.

For large-scale epidemiological studies, where diet diaries and interviewer-based recall are not practical, the food-frequency questionnaire was developed (Wiehl & Reed, 1960). This is essentially a list of foods, typically grouped by type (e.g., vegetables, fruits, breads) and the respondent indicates how often they consume each food by selecting a frequency of consumption category (e.g., daily, weekly, etc.). This method is usually employed on current diet but has also been applied to a person's historical diet (e.g., as a teenager). It has also been widely adopted outside of nutritional epidemiology, and there are now a number of different standardised food frequency questionnaires, which are often tailored to particular countries (e.g., Australian Eating Survey) or to a particular type of diet (e.g., Western-style diet). This approach often does not collect information about the quantity of food consumed, but there are variants that do.

Dietary data collected by food frequency questionnaires positively correlate with intake data obtained using diet diaries and 24-hour recall (Willett et al., 1985). Participants may also be more accurate at recalling consumption frequencies than the specific details needed for a 24-hour recall (Smith, 1993). Food frequency questionnaires have the major advantage that they are quick, do not intrude on day-to-day life (like diaries), prompt for foods of interest, minimise socially desirable answering (i.e., no interviewer present) and provide a broader timeframe than is available from a detailed study of just a few days.

One of the reasons people are concerned about the validity of all forms of self-report diet data is because of a robust finding indicating under-reporting of food intake (e.g., Fries, Green, & Bowen, 1995; Schoeller, 1995). This has been revealed by the doubly labelled water technique, which allows for an accurate assessment of energy expenditure in people as they go about their normal lives (it requires ingestion of isotopes of hydrogen and oxygen, and measurement of their excretion, so it remains a specialised test). A typical finding is that energy expenditure measured

with this technique is higher than energy intake from self-report measures (Livingstone & Black, 2003; Schoeller, 1995). The implication of this discrepancy, often in the order of 20%, is that participants routinely under-report what they eat. A number of studies have examined for systematic variation in under-reporting. The results are not that surprising. Individuals who may feel embarrassed about what or how much they eat tend to under-report the most (e.g., dieters and overweight/obese participants; Tooze et al., 2004). There may also be more systematic reasons, such as memory errors. One example is snacking. Snacking is often not anchored to daily time routines like meals, making recall more problematic, and contributing to under-reporting.

One way to deal with the problem of under-reporting is to mathematically estimate energy requirements based on a person's weight, age, gender, etc. and then exclude their diet data if there is more than a certain level of discrepancy between their reported and expected values. This method is probably necessary if one is interested in individual-level nutrients, especially if comparisons depend on absolute levels of intake or measures relative to overall energy intake. Another approach is to focus on the general dietary pattern, rather than on absolute intake values (Newby & Tucker, 2004; Newby et al., 2006). A lot of studies have started to adopt this approach, either using statistical techniques to identify stable groups of participants with similar dietary patterns a posteriori (e.g., Dekker et al., 2013) or identifying groups based on a priori criteria – typically if it is a healthy diet of one kind or another (e.g., Kant, 1996). The pattern approach has a lot of ecological validity, as people generally eat *whole* diets that share many similarities to others in their culture, country, age and socio-economic group.

A further issue concerns the long-term stability of a person's diet and how it changes across the lifespan. When reliability is measured for a dietary reporting technique, the time span is usually weeks or months, and while there might be some changes over this period (e.g., dieting, illness, travel), one would expect – and typically find – stability. However, many studies are interested in much longer time periods, years or decades. There is a growing amount of data on this. With adult diets over a 10-year period, around 30–40% of people stick with a particular dietary pattern (e.g., Dekker et al., 2013; Pachucki, 2012). Logically, this means that some 60–70% change their dietary pattern, which for epidemiological work is troubling if you want to use diet data at one point in time to predict something at a later point in time (troubling if you *assume* continuity of diet, that is). Across the lifespan, diet changes rapidly during child

development, as an infant moves from milk to a growing range of foods and thence to an adult-like diet. There is, however, plenty of evidence that diet can be adequately measured in children (e.g., Lioret et al., 2015).

Two other diet measurement approaches are available, but these are usually used to validate self-reports. The first are observational methods, either covert or overt, where aspects of a person's food habits are observed or recorded. This can include what they buy in a supermarket, shopping receipts, their choices in a canteen, their choices in closed settings (e.g., 1 week in a live-in experiment) or an examination of their kitchen cupboards (e.g., Hise et al., 2002). The second is to use biological markers (biomarkers) of diet. There are several available. The easiest to administer is reflectance spectrophotometry, which involves a device that measurers light reflected by the skin (ideally palms of the hands or inside forearm). The greater the level of carotenoids in the skin, the yellower its colour, which is accurately detected by the spectrophotometer. Diets that increase fruit and vegetable content increase skin yellowness irrespective of skin pigmentation (e.g., Tan et al., 2015), and thus reflectance spectrophotometry provides a rapid means of validating dietary self-reports of fruit and vegetable intake. Other biomarkers involve sampling urine or blood, and less frequently saliva, hair or faeces. Urine can be used to establish dietary protein intake, and the amount of added sugar in a person's diet (Tasevska et al., 2005). It is necessary to determine urine osmolarity and hence dilution, so that samples can be standardised for comparison. Bloods can provide a number of measures of fat intake either in plasma or in erythrocyte membranes. These provide data on fatty acid exposure either immediately (plasma) or over the last week (erythrocyte membrane). Importantly, these fat measures reflect the ratio of different types of fat in a person's diet (Patel et al., 2010) and not the absolute amounts consumed. Vitamin and mineral levels can also be obtained from such samples, but these have been less often used for diet validation.

Measuring diet has its limitations, and so an obvious alternative is to shift from a correlational approach to an experimental one and manipulate diet directly. This is the main-stay of animal models and is one reason why they are of interest to human researchers. In humans, compliance (i.e., are they eating what they are supposed to be?) is the main methodological problem of diet intervention studies. One approach is to feed participants for a set period of time wholly within the laboratory, but this is expensive and typically can only be done with small numbers of participants, as it is time consuming and disruptive for those taking part. A compromise is to have participants eat part of their intervention diet under controlled

circumstances, thus ensuring that the key part of the manipulation occurs. Nonetheless, it is still necessary to know what else they ate outside of the lab. For studies that leave it up to the participant to enact changes in 'real life' (e.g., eat more fruit and vegetables), how does one ensure compliance? There are no magic solutions, and the usual approach is to adopt multiple measures. These might include using their smart phone to take photos of meals/snacks, bringing back shopping dockets under the guise of reimbursing them for food, using diet diaries and interviews and spot-contacting participants by text/phone.

Dietary interventions outside the controlled setting of a laboratory often have a bad reputation. This is because they are conflated with self-performed unsupervised *diet* interventions, where the aim is to reduce energy intake and lose weight. Such energy-restricting diets are hard to comply with as it is difficult to forego highly palatable foods, endure cravings and feel hungry. While most participants start well, as weeks pass, compliance becomes a major issue. In contrast, when participants are motivated to make dietary change (e.g., in studies on diet and mental health/cognition), when this occurs as part of a study, when the changes are not excessively restrictive and are for defined periods of time (e.g., a couple of days or weeks), compliance may be no different than for other 'lifestyle' interventions (e.g., therapy, exercise, meditation).

1.4.2 Brain and Mind

As the primary focus of this book is on human data, most of the material outlined in this section relates to people. (For animal studies, relevant methodological issues are dealt with as they arise in subsequent chapters.) The main human approaches involve anecdotal reports, single case designs (one person contrasted to a control group), correlational studies and experimental designs. For anecdotal reports we have included these in a few cases, for two reasons. First, some situations are so extreme that the only available data is anecdotal (e.g., famine). Second, anecdotal information has value for generating hypotheses for more formal study. For single case and correlational designs, it is not possible to establish causality and for this we need experimental studies. One important issue to be aware of with experimental methods here is the shading into the approaches used in psychopharmacology. Some psychopharmacological approaches do not always readily translate into our field, especially utilisation of placebos and blinding, which can be difficult to enact in some circumstances (Lieberman, 2007).

For dependent variables, several types reoccur. Many studies have included systematic behavioural observations, with the Ancel Keys Minnesota Starvation Study being the stand-out example (Keys, 1946). There are many methods that use participant self-report. These may include use of questionnaires (some of which are standardised and highly reliable, such as those of personality dimensions like the 'big 5') and structured interviews. In many cases the dependent variable is of a special sort – neuropsychological tests. These first emerged in the early twentieth century, as a means to reliably and validly measure aspects of general cognitive function. The initial applications were in education, with pioneering work by Sir Cyril Burt in the UK using intelligence tests to identify children with special needs, and in the military in the United States by Lewis Terman, to find recruits suitable for officer training programs. From these beginnings progressively more and more tests of general and specific cognitive function have been developed. Modern test compendium's like Lezak et al. (2012) now feature more than 800 different tests.

The majority of these tests are designed to detect impairments in people with some form of brain injury, by providing a score relative to a healthy and often age, gender and educationally matched normative sample. These tests, especially those that are commercially available, have the benefit of being well designed, reliable (i.e., good test-retest reliability over 1 month, good internal reliability) and valid. Validity may include demonstrable linkages between impaired performance and damage to a particular area of the brain purported to underpin the measured function (e.g., memory-hippocampus; neuroimaging is also important here; Sperling et al., 2003), to functional correlates (e.g., spouse notes person has many memory lapses in day-to-day life – such data is rarely present for many tests) and to other tests that claim to measure similar abilities (this being a common form of validation).

There are several weaknesses of neuropsychological tests as applied to their use in studies examining impacts or correlates of diet and cognitive function (beyond issues of sensitivity *in healthy samples* – more later). The first is that there are so many tests, with many having been used once or twice in the nutrition-behaviour literature (de Jager et al., 2014). Consequently, it can be difficult to know which to select. Some important considerations are: (1) whether the test is in the public domain or whether it has to be purchased; (2) whether it has been employed before in a similar study to good effect; (3) whether it is being used in large-scale scientific enterprises (e.g., UK Biobank); (4) whether it is recommended by experts

in the field (e.g., de Jager et al., 2014; Lieberman, 2007); and (5) if it forms part of a broader battery of established tests (e.g., CANTAB). A further issue is whether the test is to be used once or multiple times, as people often improve with practice (Collie et al., 2003). In this respect, some tests have multiple alternate forms; others do not.

Arguably the most critical issue concerns sensitivity to detect a real change in cognitive function. This is important because as described earlier, neuropsychological tests are not generally designed for measuring what *may* be rather subtle changes induced by some nutritional variable. Thus the issue of test sensitivity looms large. As a rule of thumb if the test is to be used in normal participants the mean score needs to be around two-thirds of the maximum score, so as to provide sufficient room for improvement or worsening. This can be gauged by piloting to assess the difficulty of the task in the target population. A control group guards against the effects of practice.

Neuropsychological tests are often organised into particular cognitive domains such as memory, attention, perception, language, motor performance, construction, reasoning, executive function and global cognition – with subdomains for each (e.g., see Lezak et al., 2012). There are many different classifications into domains, and how valid they are is open to question. This is important because when studies are pooled for meta-analyses or for systematic review, such domain groupings are almost universal, and the way that results are grouped can affect whether a significant effect is or is not observed. Part of the problem here is that neuropsychological tests inevitably draw on multiple cognitive abilities even if their *name* suggests they measure just one thing. For example, almost all tests require the participant to attend, and most will utilise some aspect of executive function, namely the ability to coordinate mental activity. Relatedly, because tests often draw on multiple mental abilities, they are not generally sensitive to function in one region of the brain, although this does vary between tests (e.g., certain tests of learning and memory, do seem particularly hippocampal dependent).

Over the last 50 years there has been a major change in the technology available to researchers wanting to link cognitive functions such as those measured by neuropsychological tests, to brain function. At the outset, it is important to note that these technologies generally only have meaning when *they are linked* to cognitive measures or reports. Finding that a dietary variable alters some aspect of brain function in the absence of any cognitive or behavioural correlate is often not very useful (see Coltheart, 2013, for related arguments). That said, these technologies have

been beneficial for identifying the neural correlates of cognition and the timing of mental events. Emerging approaches such as multivariate pattern analysis offer a means of testing neural representations of mental content and go beyond these other outcomes.

In terms of technologies, the one most frequently found in this book is functional magnetic resonance imaging (fMRI), where the participant undertakes a cognitive task in the scanner, with contrasts made against a suitable control task. The aim is to identify areas of the brain that are experiencing altered blood flow and which are correlated just to task performance. fMRI has excellent spatial resolution but is not so good for temporal resolution, which is where electro-encephalography (EEG) and magnetic encephalography (MEG) have their advantage. These techniques measure electrical currents and magnetic fields on the brain's surface, respectively, but are poor at localising effects in space, but good at determining the temporal ordering of mental processes. Another approach is fDG PET, but this is less often used in healthy populations as it requires exposure to radioisotopes. Newer techniques using infrared and ultrasound are not yet established in our literature.

1.5 What to Include and What Not to Include

In reading thousands of papers on diet, brain and behaviour, it becomes apparent that the literature can be crudely split into studies dealing with single dietary agents (e.g., glucose, a vitamin, a particular food (say grapes)) versus those dealing with dietary patterns (e.g., a Mediterranean diet, a Western-style diet). This divide appears in both correlational studies and in experimental designs (e.g., manipulating exposure to a particular dietary agent vs a dietary pattern). In this book we have given *somewhat* greater emphasis to dietary pattern data than to single dietary agents. There are several reasons for this. As others have noted before, studies of single foods/nutrients are problematic because the normal human diet contains hundreds of chemicals, resulting in multiple complex interactions amongst these constituents (Newby & Tucker, 2004; Newby et al., 2006). Thus, a finding of an effect related to or caused by one dietary agent, when so many other correlated and interacting agents are present, can be hard to interpret (although there are some very important exceptions, especially relating to vitamins and micronutrients).

As we described earlier in this chapter, the accuracy with which we can measure a person's diet is good, but it is also limited. The question we pose is this: Which is likely to be more accurate (i.e., closer to what is

objectively consumed): to characterise adherence to a particular dietary pattern or to determine intake of a particular dietary agent? We suggest *in general* dietary patterns can be assessed more accurately because: (1) they are less reliant on quantification of the amount of each food that a person eats – something that is known to be difficult to measure accurately (Schoeller, 1995; Smith, 1993), and (2) pattern data is more likely to utilise all of a person's nutritional data points, thereby increasing the internal reliability of the measure. The 'in general' was italicised as there are some important caveats surrounding the claimed superiority of dietary pattern data over single dietary agent data.

The first concerns single dietary agent studies that include biomarkers relevant to that agent. These biomarkers are likely to be either an indirect correlate of the target dietary agent (e.g., a metabolite) or a direct measure of it (e.g., vitamin, mineral). Assuming that the biomarker is lawfully related to ingestion of the particular dietary agent, and that non-dietary influences are minimal/understood (e.g., it is possible to have a micronutrient deficiency even with adequate intake because the body may miss an enzyme needed to utilise it), then these types of single dietary agent studies are methodologically robust.

If there is no biomarker data, then additional considerations come into play (noting that these apply generally). The first concerns the quality of the dietary measures, and the extent to which they provide an accurate guide to consumption of the target agent (i.e., do they conform with the sort of measurement approaches outlined earlier in this chapter and an *awareness* of their limitations?). The second issue concerns adequate sample size, a particular problem in the field of psychology and neuroscience generally, and hence here too (Szucs & Ioannidis, 2017). While large sample sizes cannot compensate for poor methodology, good methodology cannot compensate for unduly small sample size, especially when dealing with the type of effect sizes that are typical in our field (i.e., small to moderate effects, with Cohen's d's between 0.2–0.5). Third, study funding source needs to be actively considered. The medical literature has amply illustrated the consistently positive relationship between funding source, and findings favourable to that funding source (e.g., Lundh et al., 2018). Fourth, there should be reasonable grounds to expect an effect, or in Hill's (1965) terminology there should be coherence. In particular, is the finding convergent with: (1) data from animal studies, (2) how the agent could affect the brain, and (3) its other known effects in humans.

If the study of a particular dietary agent has no biomarker data and is solely reliant on a dietary report, then good diet collection methodology, a

suitably large sample size, the absence of a biasing funding source and a suitable a priori expectation for obtaining the effect are factors favourable to its validity, and hence for its inclusion in this book. If it is an experimental design using a particular dietary agent, then largely the same considerations apply, plus two additional ones. First, with regards to methodology, a key issue becomes compliance (did the participant consume the dietary agent?), and again whether any biomarker data was available to confirm this. Second, whether adequate blinding (if needed) and control conditions (e.g., placebo) were instigated to ensure that attribution of an effect to the dietary agent is the correct conclusion (Lieberman, 2007).

With dietary pattern data, we suggest that because it is possible to measure this more accurately, there is likely to be less error variance with this measure. This makes studies using dietary pattern data *somewhat* less susceptible to the biases identified before. However, it will not rescue a poorly designed, low-powered study with little a priori scientific expectation of finding a particular effect (and noting the subjectivities that are involved in data-driven dietary pattern analyses, see Newby & Tucker, 2004). Dietary pattern data is not an invitation to abandon scientific standards, it just offers a somewhat more accurate indication of what a person is consuming, as well as better reflecting what people actually do – eat combinations of foods with all their complex interactions (Kant, 1996; Newby & Tucker, 2004; Newby et al., 2006). It is for this reason that we treat dietary pattern data somewhat more favourably.

CHAPTER 2

Pregnancy, Infancy and Development

2.1 Introduction

Nutrition during early life is important because it affects neurodevelopment not just acutely, but with potentially long-term consequences into childhood and adulthood. The majority of brain development occurs during the perinatal period (see Figure 2.1). The brain begins to form approximately 2 weeks from fertilisation, and reaches 80% of its adult size by 2 years of age (Lagercrantz, 2016). The growth rate of the neonatal brain is among the highest during the lifespan, with a rapid rate of neuronal and glial growth, and establishment of complex structural connections (Lagercrantz, 2016). The brain has high energy demands during this period, consuming 60% of the body's oxygen (Kuzawa, 1998).

The perinatal period begins from fertilisation, and lasts through gestation and lactation until weaning. In utero during gestation, the foetus receives vital nutrients through the placenta, which regulates their exchange and that of oxygen, as well as serving as a barrier to control which maternal endocrine and immune factors reach the foetus. After birth during lactation, the infant receives breast milk from the mother or supplementation with formula as a primary form of nutrition. During weaning, the infant transitions to solid food, and gradually adopts a more adult-like dietary pattern through childhood. Diet, supplying both energy and nutrients, is therefore an important environmental factor on brain and cognitive development. In this chapter we discuss the effects of breastfeeding, weaning, maternal starvation, starvation during infancy and early childhood, its remediation, maternal and child overnutrition, and specific nutrient and micronutrient deficiencies and their supplementation on brain and cognitive development.

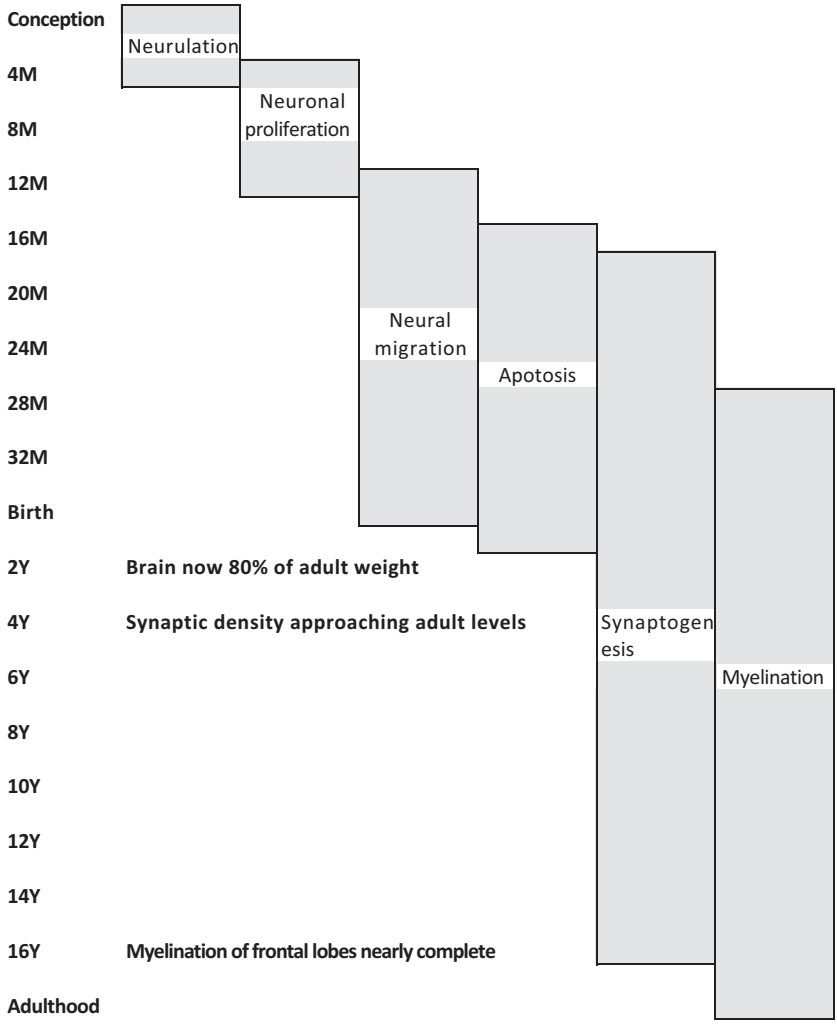

Figure 2.1 Development of the human brain

2.2 Breastfeeding

2.2.1 The Neurobiology of Breastfeeding

Breastfeeding is argued to have many benefits, including improved neurodevelopment. There are several mechanisms through which breastfeeding

could improve brain function of the child. It provides the infant with a nutritionally complete diet, which also includes proteins that assist the development of immunity to respiratory infections. There are also several constituents of breast milk that are thought to confer benefit, such as essential long-chain polyunsaturated fatty acids (lcPUFAs). In this regard, docosahexaenoic acid (DHA) and arachidonic acid (AA) have been of particular interest as they are essential for the development of the central nervous system and are found in greater concentrations in human breast milk than in infant formula (Koletzko et al., 2001). Breastfed infants have higher concentrations of these fatty acids that are positively associated with brain development (Farquharson et al., 1992; Isaacs et al., 2010).

Rather than nutritional factors, however, the intimacy of breastfeeding may facilitate the development of the infant–mother bond, and provide a soothing environment in which the infant can feed. Breastfeeding promotes release of oxytocin for the mother, can benefit mother's mood and helps mothers bond with their child, therefore indirectly affect the child's neurodevelopment through environmental enrichment.

Breastfeeding is now strongly promoted both by the American Pediatrics Association and by the World Health Organization. Although some of this drive to get women breastfeeding comes from the clear benefit in not exposing infants to formula mixed with pathogen-laden water, a significant component of the argument in favour of breastfeeding, especially in the developed world, comes from the putative long-term benefits that it confers. These benefits appear to be extensive, spanning lower body mass index in adulthood, reduced rates of asthma and eczema, and improved neurodevelopment, including academic performance, intelligence quotient (IQ), mental health outcomes and neuroanatomical differences. The evidence for impact on neurodevelopmental outcomes is discussed next.

2.2.2 *Breastfeeding and Cognition*

2.2.2.1 *Observational Studies of the Relationship between Breastfeeding and Cognition*

Numerous studies report that breastfeeding is associated with improved cognitive outcomes in childhood. The majority of studies undertaken on this subject are observational.

A meta-analysis of articles published between 2007 and 2013 identified 17 studies investigating the relationship between breastfeeding and performance on intelligence tests. All studies showed a beneficial effect of

breastfeeding on intelligence tests, with breastfed children showing a mean of 3.44 points higher on intelligence tests (95% confidence interval: 2.30; 4.58). The authors note, however, that studies that controlled for maternal IQ showed a smaller effect of 2.62 points.

Since that meta-analysis, a few additional studies have been conducted. We have reported here only those that control for relevant confounding factors such as parental education or maternal intelligence quotient in their analysis. Julvez et al. (2014) examined exclusive breastfeeding compared to no breastfeeding, for differing durations (<4 months, 4–6 months and >6 months). After controlling for maternal IQ, as well as for other sociodemographic factors, strong positive associations were observed with neuropsychological development in children at 4 years of age, particularly amongst the group who was breastfed for >6 months. Belfort et al. (2016) followed 1,037 participants from birth to 7–10 years of age. Executive functions and social-emotional functions were assessed using self-report measures completed by parents and teachers. Neither were found to differ according to either breastfeeding duration or exclusive breastfeeding duration. Breastfeeding duration in 926 Korean children predicted cognitive development, as well as internalising (e.g., worry) and externalising (e.g., aggression) problems at 5-years of age (Kang, 2017). A further report suggests the effects of breastfeeding persist into old age, with a study of 931 men born in 1934–1944 in Finland showing that breastfed men had higher cognitive ability. Longer duration of breastfeeding predicted cognitive ability scores, when tested at both 20.2 years and 67.9 years (on average) of age.

Overall, most of these observational studies show that breastfeeding is associated with higher scores on cognitive tests. However, these findings do not demonstrate causality as they are confounded by sociodemographic factors that are simultaneously related to infant feeding practices, as well as long-term child outcomes. Studies show that compared with bottle-fed infants, infants that are breast fed tend to (1) be born into families with higher socioeconomic status; (2) have higher parental educational attainment; (3) have easier access to healthcare services; (4) live in areas with less exposure to environmental toxins; (5) have mothers that are less likely to have smoked during pregnancy; and (6) have carers with parenting attitudes or styles more favourable to successful development (Rothstein, 2013; Singh, Kogan, & Dee, 2007; van Rossem et al., 2009). Duration of breastfeeding is also associated with these factors, as well as a mother's psychological state 4 years postnatally (Julvez et al., 2014). While observational studies typically attempt to statistically control for some of these

factors, it is difficult to control for all heterogeneity, particularly unobserved heterogeneity. As such, comparison of breast- and formula-fed infants is likely to observe outcomes more favourable to those who have been breastfed.

2.2.2.2 *Experimental Studies Investigating the Effect of Breastfeeding on Cognition*

Though it is difficult ethically to conduct an experimental design assigning mothers to breastfeed or formula, a few studies have employed naturalistic experiments or experimental manipulations. Four studies have used sibling comparisons to separate the impact on cognition and behaviour of factors that predict a mother's selection to breastfeed from the actual consequences of breastfeeding. By comparing outcomes of siblings who were fed differently in infancy, it is possible to estimate what the bottle-fed sibling's outcomes might have been.

Der, Batty, and Deary (2006) compared the academic achievement of differently fed siblings and found no impact of breastfeeding status (yes vs no) or duration of breastfeeding. Evenhouse and Reilly (2005) examined the relationship between breastfeeding and 15 different outcomes related to physical health, emotional health and cognitive abilities. Using the typical between-family model, all 15 correlations were significant. However, when using a within-family model to determine if the differences in outcomes between siblings were correlated with different breastfeeding histories, all but one correlation became non-significant. The one remaining significant correlation was with the Peabody Picture Vocabulary Test, a measure of verbal intelligence. However, it is notable that the children did not differ on other cognitive measures – grade point average across maths, science, social studies, language and arts; history of repeating a grade; or whether the child reported being "highly likely" to go to college. In terms of mental health, there was no significant relationship between depression symptoms, mother or child report of closeness, how strongly the child agreed that the mother is usually warm and loving, or the range of activities in which child and mother participate each month.

Using a similar design, Colen and Ramey (2014) examined 11 different outcomes in children who were aged 4–14 years old, including body mass index, obesity, asthma, hyperactivity, parental attachment, behavioural compliance, reading comprehension, vocabulary, math ability, working memory and academic skills. When the full sample was analysed, findings suggested that children who were breastfed during their first year of life had better outcomes on all variables except asthma. However, when

limiting comparisons to within, rather than across families, none of the effects remained significant. Again, with a similar design, Rothstein (2013) showed that breastfeeding for 6 months or more was associated with better cognitive outcomes in children aged 5–6 years. However, when comparing within families, there was no statistically significant effect. Thus, overall, the sibling studies show that the observational findings are overestimating breastfeeding benefits, and, with the exception of one significant relationship between verbal intelligence and breastfeeding, a large number of correlations between physical, emotional and cognitive outcomes appeared to be due to unobserved variation between families that leads to bias in selection to breastfeeding, rather than a causal outcome of breastfeeding itself.

While sibling comparisons are a powerful method for reducing between-family selection bias, they do not control for difference that might occur within families. That is, there are some factors that contribute to why one sibling might be breast fed, whereas another is bottle fed. Two studies have been conducted that have performed experimental manipulations that have increased the amount of breast milk in the infant diet. The first, conducted by Isaacs et al. (2010), included mothers who elected to breastfeed, but for varying reasons were unsuccessful in doing so and therefore their infants were bottle fed. Study infants were randomised to receive either preterm formula (PTF; $n = 28$), standard infant formula (SIF; $n = 13$) or banked breast milk (BBM; $n = 9$). Percent exclusive breast milk (%EBM) in the infant diet was found to be positively correlated with verbal IQ in adolescence (average age 15 years, 9 months). In turn, verbal IQ was associated with white matter volume, whereas no association was seen with grey matter volume. Notably, this study was performed in mothers who had chosen to breastfeed and there was no relationship between either social class or maternal education and %EBM. Furthermore, maternal IQ was no different between those mothers who tried to provide breast milk but failed and those who did not try to provide breast milk. This does seem to suggest that there is a difference in cognitive function in those infants who actually received the breast milk, rather than pre-existing differences in those who chose to breastfeed.

There is only one randomised controlled trial (RCT) in this area. Kramer et al. (2008) compared two groups: one that received breastfeeding promotion and education, the other receiving no education. The educational program increased the rates of exclusive breastfeeding at 3 months in the experimental group (43.4%) compared to the control group (6.4%). When

children were tested at age 6.5 years, a higher overall intelligence quotient (IQ) of about 5.9 IQ points was observed for the Education group compared to the Control group. Note, however, that this is only 0.6 of a standard deviation of the population IQ distribution. Subsample analyses showed that the advantage of breastfeeding was around three IQ points for children born at term and about five IQ points for children born pre-term. While these are moderate effects in population terms, at the individual level a difference of three IQ points is unlikely to make a large difference over and above other environmental factors. Furthermore, the paediatricians who administered the neuropsychological tasks were not blinded to the condition of the subjects, and some of the scales' scoring criteria can be subjective. In particular, the verbal subscales are more subjective in scoring than non-verbal, and there was a greater effect found for the verbal than non-verbal subscales. To compensate for the non-blinding of the paediatricians, an audit was undertaken whereby one in five of the participants underwent repeat testing by a clinician who was blind. While a significant effect was still obtained in this smaller sample, it was of a smaller magnitude, of about three IQ points. Furthermore, teacher ratings of academic achievement were obtained for 75% of the children, and showed that across four domains (reading, writing, mathematics, other), although ratings were slightly higher in the experimental group, the 95% confidence intervals included 0, indicating non-significance. It should be noted, however, that the nature of the experimental design would likely underestimate the causal effect of breastfeeding due to the substantial overlap in rates of breastfeeding in the educated versus control groups (although maternal IQ might be predictive of a greater response to the educational manipulation). Therefore, the study does provide some evidence of a causal relationship between breastfeeding and IQ; however, the magnitude of the true effect remains unknown.

What does seem to be clear from this body of research is that the risks associated with a failure to breastfeed appear to be overestimated in the observational literature. This is an important point because there is often significant pressure for mothers to breastfeed, and it can also be a stigmatising decision to switch to formula feeding if breastfeeding is unsuccessful. This can alienate mothers and can result in stress and anxiety, which in turn have an effect on developmental outcomes of the child. Based on the available evidence, mothers should be encouraged to breastfeed, and supported in their ability to do so. However, they should also be reassured that should they be unable to do so, that the adverse consequences are likely to be minimal in terms of cognitive development.

2.2.2.3 *Breastfeeding and Cognition in Preterm Infants*

As with the literature examining breastfeeding and cognition in term infants, most of the studies on preterm infants have been observational. Of those that are of adequate methodological quality and control for confounding factors such as maternal IQ, none found any difference between breastfeeding and formula feeding (Elgen, Sommerfelt, & Ellertsen, 2003; Pinelli, Saigal, & Atkinson, 2003). Furthermore, a re-analysis of data from a randomised controlled trial comparing breastfeeding with a formula enriched with arachidonic acid and docosahexaenoic acid showed no differences in cognitive development between the groups. A Cochrane review of formula feeding compared to breast milk feeding in preterm infants identified four trials that have assessed neurodevelopmental outcomes in children aged at least 12 months, measured using validated assessment tools, and overall found no evidence for effects on neurodevelopmental outcomes (Quigley, Embleton, & McGuire, 2018).

2.2.3 *Breastfeeding and Mental Health Outcomes*

Mental health outcomes in this section refer specifically to the child. While there is a substantial literature regarding the relationship between breastfeeding and mental health of the mother, this is not related to nutritional intake per se. Overall, while there are some studies that have observed that breastfeeding is associated with fewer psychosocial difficulties in childhood and adolescence (Heikkila et al., 2011; Julvez et al., 2007; Liu, Leung, & Yang, 2014; Oddy et al., 2010), most others have observed that these associations are no longer significant after adjusting for the many potential confounding factors that were identified earlier in this chapter (e.g., Lind et al., 2014; Waylen et al., 2009). Importantly, in the one RCT of a breastfeeding promotion intervention (as described in Section 2.2.2.2), no difference between experimental groups was found for psychosocial measures.

2.2.4 *Breastfeeding and Brain Development*

The cognitive findings described so far are complemented by neuroimaging and psychophysiological findings, although these too are affected by exactly the same confounds that dog interpretation of the cognitive data. Analysis of evoked potentials in 1-year-olds (as a measure of neural maturation) showed greater wave latencies in the visual and auditory pathways of formula-fed infants, suggesting delayed myelination of these

pathways compared to breastfed infants (Khedr et al., 2004). In 133 healthy children aged 10 months through to 4 years, those who were exclusively breastfed (n = 85) exhibited increased white matter volume in late maturing white matter regions, compared to the formula-fed (n = 38) and mixed breast- and formula-fed (n = 51) groups (Deoni et al., 2013). Longer breastfeeding duration was also associated with a more intact white matter microstructure. Notably, there were no significant differences between breastfed, formula-fed and mixed feeding groups on potential confounding variables (age, gender, birth weight, gestation duration or maternal age, education or socioeconomic status). In 571 adolescents aged 12–18 years, breastfeeding was a significant predictor of cortical thickness in the parietal lobe, controlling for other relevant predictors such as parental education (Kafouri et al., 2013). In the randomised feeding trial described (Isaacs et al., 2010), brain imaging demonstrated that higher percentage expressed breast milk consumption was associated with greater total brain volume and white matter volume in adolescents (average age 15 years, 9 months). Furthermore, white matter volume was correlated with both verbal and full-scale intelligence quotients, suggesting that white matter development may underlie the effect of breastfeeding on cognition. This is turn may be linked back to differences between formula and breast milk in lcPUFAs, which are important to myelination.

2.3 Weaning and Consequences of Lack of Exposure to Solid Food

All mammals move from suckling to the separate processes of drinking fluids and eating solid foods (Bond et al., 2020). Humans are unique amongst mammals in that weaning occurs before the offspring is capable of independently obtaining their own food. In pre-industrial cultures, mothers start introducing solid foods at around 4–6 months, with breast milk consumption faded out over a 30-month period (Borowitz, 2021). While there is considerable cultural variation, this general pattern tends to hold true in developed countries too. There also appears to be a window, less than 12 months and greater than 3 months, in which the infant is particularly receptive to new flavours and textures. Missing this window by delaying the introduction of solid foods beyond 12 months is reportedly associated with a greater likelihood of feeding problems into childhood, and with less tolerance for textural variety – vegetables and fruit in particular (Borowitz, 2021).

Several authors have observed that once a baby has been tube fed – perhaps because they were preterm or failed to thrive – and when the tube feeding stops, a large proportion (over two-thirds) seem to have major problems transitioning to oral feeding with either milk (from breast or bottle) or to solid food (Avitzur & Courtney-Martin, 2016; Miller, 2009; Wilken et al., 2018). The suggestion is that infants in general have to: (1) learn that the sensations of food in the mouth and swallowing are associated with the cessation of hunger and the rewarding development of satiety; (2) become familiar with the unusual sensations of fluids and foods in the mouth and oesophagus; and (3) learn how to swallow, and practice this repeatedly so that appropriate aerodigestive coordination develops (Miller, 2009). It seems that if this process is delayed, as occurs with tube-fed babies, the more difficult it becomes to get the infant to feed orally.

2.4 Malnutrition: Impacts on Foetal and Child Development

2.4.1 Introduction

In infants, malnutrition may result from either an energy-deficient (manifesting as Marasmus) or protein-deficient (manifesting as Kwashiorkor) diet – or more commonly both (protein-energy malnutrition, or PEM). The effects on development of all forms of malnutrition are profound. Its most evident physical manifestation is stunting, namely a reduced height for age, formally defined as a height for age more than 2 standard deviations below the norm. Stunting affects around *150 million children under the age of 5* (World Health Organization, 2020). Moreover, malnutrition is responsible for between 3 and 5 million deaths in children under 5 each year (i.e., conservatively 1 child dying every 10 s), with most stunting and associated mortality concentrated in sub-Saharan Africa and Asia (Black et al., 2008; Collinge et al., 2006). This section examines the impacts of malnutrition during pregnancy on the foetus in animal models and humans, the effects of stunting on child development, and its remediation. As many authors regard overnutrition and poor diet quality as also being aspects of malnutrition, we also examine the impact of these during pregnancy and on the developing child in this section.

2.4.2 Foetal Development and Models of Dietary Impact

Nutrition during the perinatal period is thought to impact brain structure and function throughout the lifespan, well beyond any periods of

deficiency. There are two basic models proposed to account for why early nutrition may have such long-term consequences (see Georgieff, 2017, for review). The first is the critical period hypothesis. A critical period can be defined either as the point of maximal brain growth or the point(s) at which fundamental organisation of brain structure is taking place – this distinction can be important because in rats maximal brain growth occurs *postnatally* (Levitsky & Strupp, 1995). The critical period hypothesis proposes that at these point(s) the brain has high nutritional requirements (Rice & Barone, 2000). Deficiencies during a critical period result in structural brain changes that may not be reversible by remediation of the deficiency after the critical period has passed. This theory is supported by findings that early deficiencies in several nutrients (long-chain polyunsaturated fatty acids (lcPUFAs), iron, zinc, iodine) can result in long-term dysfunction into childhood and adulthood (Georgieff, Brunette, & Tran, 2015).

The second model is the altered-regulation hypothesis, which suggests that early nutrient deficiency results in epigenetic changes. That is nutrient status is an environmental factor that can alter gene expression with long-term consequences on the central nervous system (Moody, Chen, & Pan, 2017). These two models are not mutually exclusive and both are likely to represent pathways by which dietary deficiencies can cause long-term impacts on the brain.

2.4.3 Maternal Starvation: Animal Data

Animal models have been used extensively to examine the impacts of maternal malnutrition on foetal and infant development, but less frequently so into adulthood (Levitsky & Strupp, 1995). Findings can be organised into the general effects of malnutrition, where an inadequate diet is provided, and more specific studies that separately model protein- and energy-deficient diets. The most general effect of malnutrition during pregnancy on the foetus is reduced brain size. This is accompanied by increased cortical cell packing, a reduction in brain myelin, greater numbers of mitochondria in cortical cells, alterations in brain metabolism and in the production and turnover of neurotransmitters and their receptors, and extensive abnormalities in the hippocampus and cerebellum. Some of these effects seem reversible (Levitsky & Strupp, 1995), while others seem to persist into adulthood, although their functional consequences are not always apparent on behavioural testing at that point in time (e.g., could these abnormalities confer vulnerability in old age for neurodegenerative disease?).

For protein-deficient diets, a normal rat chow will deliver approximately 15–20% protein by weight, and restricted diets for pregnant dams may reduce this to as little as 6%. The consequences for foetal brain development are significant, and while a lot of parameters vary between studies (e.g., duration of maternal low-protein diet, protein concentration) the basic effects are well understood (Barra et al., 2018). Abnormalities to the hippocampus, cerebellum, cortex and basal ganglia are usually observed, along with alterations in neurotransmitter levels in particular structures, such as dopamine reduction in the pre-frontal cortex, and an increased propensity for oxidative stress in the hippocampus and cerebellum (Alamy & Bengelloun, 2012; Morgane, Mokler, & Galler, 2002). In the developing rat, these brain deficits manifest in abnormal behaviour (Barra et al., 2018). There are changes to exploratory, social and emotional activity; abnormal sleep-wake cycles; impairments to hippocampal-dependent learning and memory (HDLM); and inhibition. Just as gross structural deficits do not seem evident in adult rats following maternal protein deprivation, behavioural deficits do not seem to continue either (Alamy & Bengelloun, 2012). This may often reflect the impact of normal levels of nutrition postnatally, suggesting some degree of recovery.

Models of calorie restriction during pregnancy cut the pregnant dams energy intake anywhere from 25–75% of what it would normally be. The effects seem more specific, with notable impacts again on the hippocampus, a smaller corpus callosum (suggesting abnormal myelination) and increased hypothalamic–pituitary–adrenal axis activity, stress reactivity and elevated levels of corticosterone (Barra et al., 2018). Behaviourally, there are abnormalities on some tests of HDLM, as well as increased impulsivity, greater reward sensitivity and socialisation deficits. The neuroanatomical effects do not seem to persist into adulthood, and the evidence for behavioural persistence is mixed (Alamy & Bengelloun, 2012). Again, this may reflect study designs favouring normal postnatal diet, thereby allowing some recovery. In many situations where human maternal malnutrition occurs (i.e., in extreme poverty), adequate postnatal diet, and hence recovery, may not be possible.

Malnutrition during pregnancy has a further legacy for the offspring (Smith & Reyes, 2017). They are at greater risk for obesity and type II diabetes than those raised in a healthy foetal environment. They also prefer and more readily consume foods rich in fat and sugar, which is probably due to dopaminergic hypofunction in brain reward areas. That is these animals seek our more potent rewards to compensate for reduced dopamine release. One way of interpreting this pattern of biobehavioural

changes is the thrifty phenotype model (Hales & Barker, 1992), in which malnutrition in the foetal environment shapes the organism, probably via epigenetic changes, towards a postnatal environment in which food is scarce. In this scarcity environment, storing excess fat, eating whenever food is available, and especially so if it is indicative of energy (i.e., sweet and fatty), and maintaining a higher resting blood glucose level are arguably adaptive. The problems emerge, however, if the foetal environment does not match the actual postnatal environment, in which case the thrifty phenotype lends itself to overeating and metabolic syndrome.

A further and related question is whether malnutrition produces any adaptive shift in behaviour paralleling the thrifty phenotype – namely foraging, exploring and risk-taking so as to maximise the chance of obtaining food – in contrast to a more risk-averse, less active and more anxious phenotype, which might be associated with a well-fed foetal and postnatal environment. Besson et al. (2016) undertook a meta-analysis of relevant rodent pregnancy malnutrition studies, but found no evidence favourable to this hypothesis. The only consistent finding was a tendency for *reduced* activity in the offspring of calorie-restricted dams.

Finally, animal studies have explored possible mechanisms by which maternal malnutrition can affect brain function. Proinflammatory factors have been shown to mediate the increased risk for models of neurodevelopmental disorders in the offspring of rat dams with 50% reduced caloric intake during pregnancy (Shen et al., 2008). In an animal model of foetal malnutrition – intrauterine growth restriction – epigenetic changes have been observed (i.e., consistent with the altered-regulation hypothesis), such as disrupted hippocampal histone methylation (Ke et al., 2014) and brain-derived neurotrophic factor (BDNF) DNA methylation (Ke et al., 2010). Maternal malnutrition may also affect the developing foetal brain through lack of specific nutrients that are critical for growth and development, including glucose, fatty acids, iron, zinc, folate, choline and several other vitamins and minerals, which are discussed later in the chapter.

2.4.4 Maternal Starvation: Human Data

Post mortem studies of severely malnourished babies, born to mothers who were malnourished during pregnancy, versus well-nourished controls show multiple brain impairments including fewer cortical neurons, shorter dendrites and abnormal dendritic spines (Benitez-Bribiesca, De la Rosa-Alvarez, & Mansilla-Olivares, 1999). Not surprisingly then, and given the animal data, one might expect significant sequelae to foetal malnutrition.

In epidemiological studies, which form all of the human data, maternal malnutrition can occur due to internal causes, such as disease (e.g., eating disorder), or from external causes, such as famine. We look at both here.

Several studies have examined internal causes, all based in developed countries. Infants born to mothers with anorexia nervosa or bulimia nervosa have reduced head circumference at birth and delayed expressive language skills when assessed at age 5. However, it is not possible to rule out confounding factors such as maternal stress during pregnancy as a cause for these developmental problems. A number of medical conditions can lead to intra-uterine growth restriction, such as hypertension in the mother, which serves to restrict blood flow to the placenta and thus to the foetus. Such restricted blood flow can result in a small for gestational age foetus, with increased risk of developmental delay, learning difficulties and lower IQ (Nyaradi et al., 2013). Maternal diabetes, with unstable blood sugar, and thus periods of hypoglycaemia (and hyperglycaemia), is linked to lower child IQ and poorer performance on several cognitive domains at age 7–11 years (Rizzo et al., 1997) – this correlated with the degree of metabolic abnormality in the mother.

Studies of underweight mothers (i.e., BMI <18) suggest that they have children who are more likely to have some form of cognitive impairment (Veena et al., 2016). Significant maternal weight loss can occur with hyperemesis gravidarum (*severe* nausea in pregnancy). Fejzo et al. (2009) selected mothers with this condition who had experienced significant weight loss during pregnancy (15% of their *pre-pregnancy* body weight). Most of these mothers, and the control mothers, were surveyed around 1–2 years post-pregnancy. Even though there was no difference in the weight of the newborn, there was a trend ($p = 0.07$) for increased rates of behavioural disorders in the extreme weight loss group (9.3% of initial body weight (IBW)), relative to controls (5.5% IBW; Fejzo et al., 2009). This may be a conservative test as many children would not have yet reached an age where behavioural disorders would be most apparent.

There are two series of studies examining the effects of famine on neurodevelopmental outcomes. The Dutch Famine Study documented the effects of a severe famine which occurred during the 'hunger winter' of 1944–1945. The German army imposed an embargo on transport to several regions in the Netherlands, with the daily ration of mainly bread, potatoes and sugar beet yielding less than 4,200 kJ. The comprehensive health records kept over this time meant that the effects on birth cohorts exposed to the famine at specific times during gestation could be compared to those who were not exposed. The offspring of mothers who had reduced