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Testing
the Protein Leverage Hypothesis
in Jamaicans and Filipinos

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Abstract

Excess energy intake is clearly implicated in the obesity epidemic, but there is uncertainty as to the role of specific macronutrients. Although carbohydrates and fat are major sources of excess dietary energy, there exists a hypothesis that protein is a key determinant of carbohydrate and fat, and hence total energy intake. The Protein Leverage Hypothesis (PLH) suggests that protein plays a key mediating role mediating in the development of obesity, through its interaction with fat and carbohydrates is not well understood. PLH predicts that humans prioritize protein when regulating food intake. In doing so, humans will consequently over-consume fats and carbohydrates when consuming diets low in protein and fats and carbohydrates will be under-consumed on diets that are high in protein. Although evidence exists in support of the PLH in two experimental studies of UK and Australian participants, and from a recent meta-analysis of experimental data, there are not data to support this hypothesis in humans living in their usual living environments.

The first aim of this thesis was to test the hypothesis that humans with a population history of severe childhood under-nutrition exhibit characteristics of the PLH. Given the growing body of work showing the importance of developmental nutritional history in predicting energy intake and obesity, it is important to determine macronutrient regulation in a nutritionally deprived population and, in particular, whether the participants exhibited protein leverage as per the PLH. The second aim of this thesis was to test the hypothesis free-living humans in a developing country undergoing the nutrition transition exhibit dietary behavior consistent with the PLH.

In this thesis, I report the first evidence in a population-level study that supports the PLH in a free-living population. Together these data contribute to the understanding of how dietary macronutrient content influences energy intake, by extending how PLH can be generalized to a population with a history of severe childhood undernutrition, and showing for

the first time that the predictions of PLH are met in population studies outside of an experimental setting. Both the population-level and experimental components of this study provide support for the role of the protein appetite in driving energy intake.

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Abbreviation

ANOVA	Analysis of variance
BMI	Body mass index
CLHNS	Cebu Longitudinal Health and Nutrition Survey
CV	Coefficient of variation
DOHaD	Developmental origins of health and disease
FAOSTAT	The statistics division of the Food and Agriculture Organization
GDP	Gross domestic product
GINI	Coefficient, measure of statistical dispersion
Kw	Kwashiorkor
MCH	Maternal-child health
Mr	Marasmus
NHMRC	National Health and Medical Research Council
NR-NCD	Nutrition-related non-communicable diseases
OECD	Organization for Economic Co-operation and Development
PLH	Protein Leverage Hypothesis
SCU	Severe childhood under-nutrition
SE	Standard error
SES	Socioeconomic status
TMRI	Tropical Medicine Research Institute
TMRU	Tropical Medicine Research Unit
WHO	World Health Organization

Chapter 1

Introduction

Dramatic changes in human diet and lifestyle are occurring globally such that physical activity is decreasing while dietary intake consists of more processed foods and less whole foods, (Popkin 2002, Eckhardt, Adair et al. 2003, Popkin and Ng 2007). This radical shift in diet and activity, known as “the nutrition transition”, may be an underlying cause of the global obesity epidemic, but the details of the relationship remain unclear. In particular, the complex interactions between food choice, food composition and energy intake in the modern diet are poorly understood. Evidence exists to suggest that the motivation for human food choices and diet composition are associated with taste, cost and convenience, and probably with specific targets for the regulation of macronutrient intake (Simpson and Raubenheimer 1997, Simpson, Batley et al. 2003, Simpson and Raubenheimer 2005, Sørensen, Mayntz et al. 2008, Brooks, Simpson et al. 2010). This introduction chapter explores dietary, physical activity and environmental shifts associated with the obesity epidemic and the relationship between these shifts and the *nutrition transition*.

1.1. The Obesity Epidemic

Obesity is defined as excess body fat accumulation that is associated with risk of chronic diseases, such as hypertension, cardiovascular disease, and type 2 diabetes (Gluckman and Hanson 2008). Obesity is caused by a chronic positive energy imbalance due to energy intake exceeding daily energy expenditure. Over the last three decades, the prevalence of obesity has increased worldwide due to diet and physical activity levels shifting towards increased energy intake and decreased energy expenditure (Hill, Wyatt et al. 2003). Due to innate human physiology it is easier to accumulate fat than to lose fat (Daniels and Adair 2004, Wells 2006). Peak fat deposition occurs early in life, during late gestation and early infancy, and again during adolescence, specifically in females (Gluckman, Hanson et al. 2007). Body fat accumulation

not only buffers malnutrition, but also regulates reproduction and immune function (Gluckman and Hanson 2004). The large capacity to deposit fat represents an adaptive feature of humans, however, depositing excess fat has become a maladaptive feature in the modern environment where fluctuations in energy supply is minor, and productivity depends on mechanization rather than physical effort (Lev-Ran 2001). The World Health Organization (WHO) projects that by 2015, approximately 2.3 billion adults worldwide will be overweight, and more than 700 million will be obese (<http://goo.gl/BnXb3>).

The increased rate of obesity was first reported in the United States and quickly spread to other developed nations before penetrating middle- and low-income countries (Popkin 2004) (Figure 1). Obesity is now becoming highly prevalent in many developing countries, particularly in urban areas (Caballero 2005). More important, low-income developing countries are now dealing with a double burden where undernutrition is still prevalent with high rates of infectious diseases as well as the increasing prevalence of obesity and co-morbidities including Type 2 diabetes, hypertension, heart disease, and some cancers (Popkin, Kim et al. 2006, Wells 2007). As the prevalence of adult obesity continues to increase, the prevalence of childhood obesity is also increasing; in 2010, the WHO estimated nearly 43 million children under the age of five years were overweight and close to 35 million of these children are living in developing countries (<http://goo.gl/kbsJgO>).

Childhood obesity seriously challenges public health systems, as overweight and obese children are likely to remain obese into adulthood and develop non-communicable diseases at a younger age (Bhargava, Sachdev et al. 2004, Cheng, Bolzenius et al. 2015). Although genetic factors may increase the risk of obesity, the environment interacts with genes to drive the increased accumulation of adipose tissues (Hill, Wyatt et al. 2003). It appears that environmental processes during the peri-conceptual, fetal, and infant phases of life influence the risk of developing diseases in adulthood (Gluckman and Hanson 2004). This view expands

the traditional study of the obesity epidemic that had previously focused on genetic and lifestyle factors as the primary determinants of fat gain.

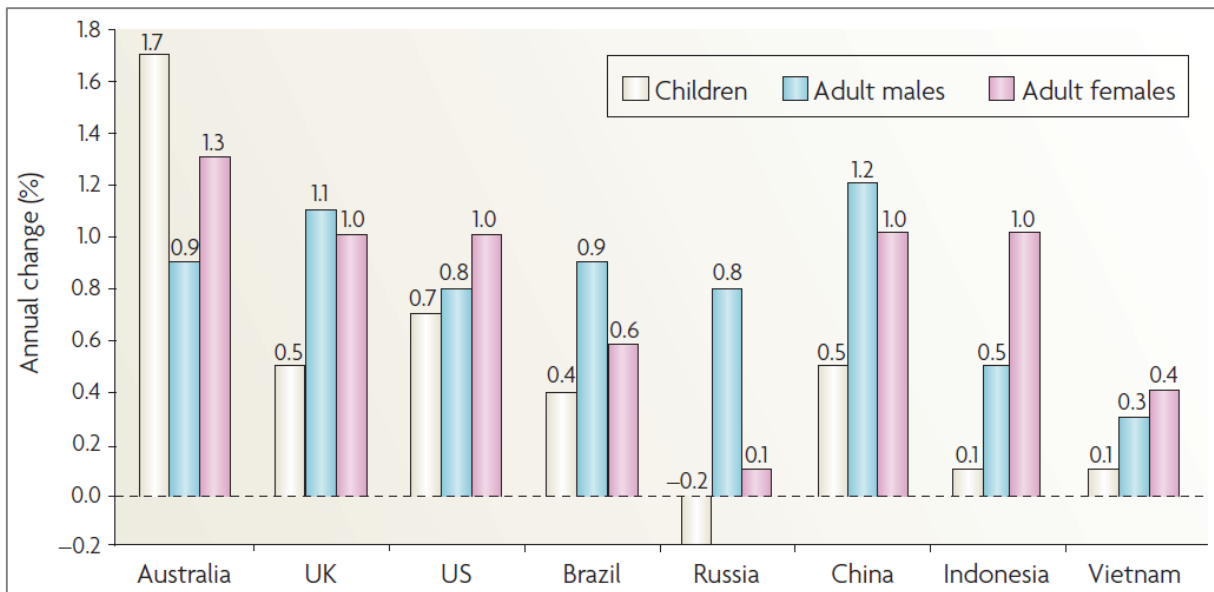


Figure 1. Annual absolute changes in the prevalence of overweight and obesity in selected countries

Data shown are the annual percentage point increases in prevalence of overweight and obesity for countries with the initial year in the period from 1985 to 1995 and the final year in the period from 1995 to 2004. Overweight and obesity is classed as a body mass index (BMI) of ≥ 25.0 kg m⁻² for adults (for children, age-gender BMI standards equivalent to adult overweight and obesity standards were used). Figure and caption from Popkin (2007).

1.1.1. Early Life Events and Obesity

Nutrition-related chronic diseases are generally attributed to genetic and/or lifestyle factors and obesity is the result of the intersection of complex, multifaceted causal pathways. The developing organism is plastic, where genetic responses to a changing environment have led to the hypothesis that disease risk is the result of complex genetic-environmental interactions. It is proposed that the inherent plasticity of an organism during windows of development allow it to respond to cues (information) that will ultimately determine the mature (adult) phenotype. Under some circumstances these developmental adaptations lead to increased disease risk (Gluckman and Hanson 2006). Adaptations in developmental processes,

specifically prenatal or early infant “programming” of metabolic pathways, may play a significant role in determining the risk of obesity and associated morbidities in a given environment. This concept, termed the “developmental origins hypothesis” suggests that normal development in humans – and all mammals – is a balance between two sets of processes: those that constrain development towards a particular phenotype (i.e. canalisation), and those that allow the phenotype to vary according to environmental signals acting during early development (developmental plasticity) without disrupting the basic developmental program (Gluckman, Hanson et al. 2005). These developmental processes could involve, at least, two pathways to disease. First, the pathway to disease would occur following a mismatch between the nutritional environment during a period of developmental plasticity that is manifested in mature phase of life. Second, adaptations may take place during periods of early-life nutritional excess (Gluckman, Hanson et al. 2005, Gluckman and Hanson 2008).

Expanding the concept of developmental plasticity, mammalian mothers (including humans) transfer information regarding the future environment to the fetus (Gluckman and Hanson 2004). This relationship has been found in several experimental and clinical studies. Vickers et al. (2000) observed an important interaction between prenatal and postnatal environments in rodents such that the consumption of a postnatal high-fat diet promoted greater adiposity in pups exposed to a maternal low-energy or low-protein environment in utero compared to those exposed to maternal high-fat diet in utero. Mechanisms to explain these findings focus on the adaptation of appetitive signalling cues, either nutrients or hormones, from the mother to the developing fetus/neonate that ultimately promote energetically thrifty phenotypes that are vulnerable to excessive energy consumption and retention when living in high-energy postnatal environments (Vickers, Breier et al. 2000, Gluckman, Hanson et al. 2007, Gluckman, Hanson et al. 2008).

Humans have gradually changed their phenotypes in response to varying energy environments over extended periods of time. It is generally accepted that obesity and its co-

morbidities, including type 2 diabetes, are manifestations of radical changes in the human nutritional environment (diet, energy intake and expenditure, relative weight, and body composition). In this regard, intergenerational cycles of obesity are evident and may be the result of a continuous relay of cues between the mother and fetus in an environment where nutritional excess predominates. Nowhere is this more prevalent than in developing countries undergoing a nutrition transition, countries emerging from a traditional diet of low-fat, high fibre to a “Western” diet high in fat and sugar. Here, pregnant women undergoing a nutritional transition may have a fetus undernourished *in utero*, but postnatal exposure to a fat-rich diet may increase the risk of obesity. These offspring are likely to enter pregnancy obese and, since maternal obesity predicts childhood obesity, the transgenerational cycle continues as obese parents extend the risk of obesity to their offspring (Gluckman, Beedle et al. 2008). In light of this theory, human populations undergoing a rapid nutritional transition would have greater risk of developing adult obesity than those human populations undergoing a more gradual transition (Prentice 2006).

In summary, evidence suggests that the mature phenotype adapts poorly to a given environment if the fetus experiences a developmental mismatch, where early life signals (i.e. low energy availability) lead the fetus to select a particular developmental pathway in an environment wholly different from the *in utero* experience (i.e. excess energy availability) (Gluckman and Hanson 2004, Gluckman, Hanson et al. 2005, Gluckman and Hanson 2008). In this regard, developmental plasticity is an important determinant of human biology as humans evolved in unstable and changing environments. The critical role of nutritional cues during early development and how these cues determine later growth and reproductive characteristics, and the interaction between humans and their energy environment, is still unclear (Gluckman, Hanson et al. 2005, Gluckman, Hanson et al. 2007). Thus a greater understanding of the role of macronutrient selection in living populations will further elucidate underlying pathways in the establishment of the obesity epidemic.

1.2. Nutrition transition and the “Modern Lifestyle”

Over the last five decades, three important human transitions have occurred simultaneously: demographic, epidemiologic, and nutritional. Demographic transitions take place in countries where the fertility and mortality pattern changes from high to low. Epidemiologic transitions correspond with a shift in the causes of morbidity and mortality from undernutrition and infectious diseases to a higher incidence of non-communicable chronic and degenerative diseases associated with the urban-industrial lifestyle (Colin Bell, Adair et al. 2002). The third process of transition refers to nutrition and includes shifts in dietary components and energy as well as changes in lifestyle (Caballero 2005) (Figure 2).

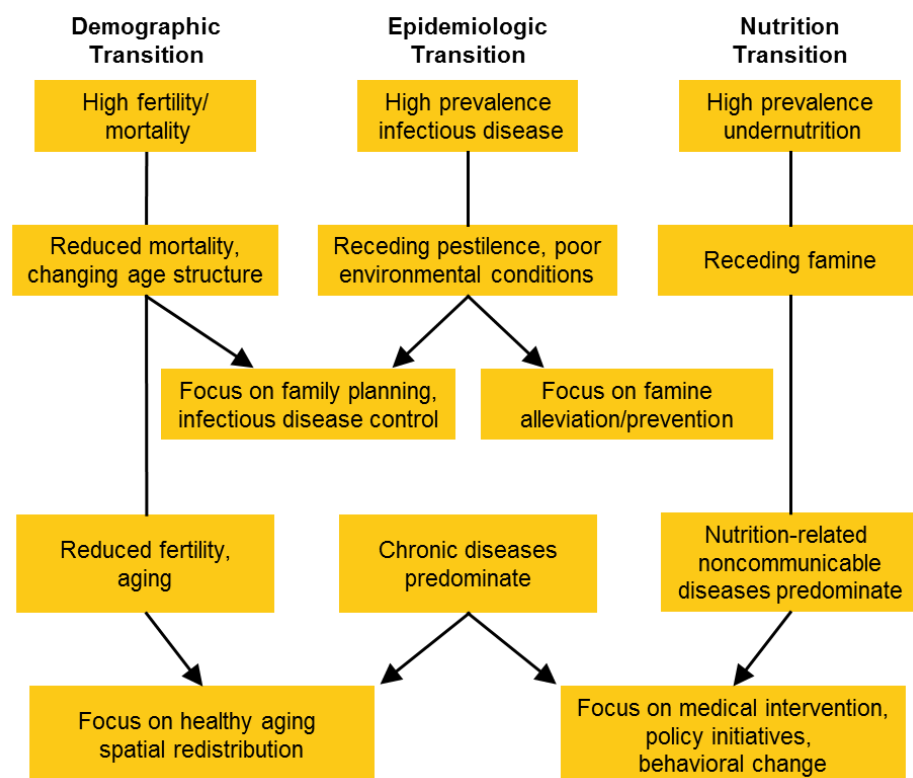


Figure 2. Stages of health, nutrition, and demographic change

Figure reproduced from Popkin (2002).

The modern lifestyle is an achievement of our ancestors, often thought of as an improvement on historical living circumstances as populations have improved nutritional

resources (including improved financial resources for increased food security), living condition with less physical labour, and increased leisure time. However, the modern lifestyle has costs, such as chronic diseases including “lifestyle associated diseases” obesity and Type 2 diabetes (Hill, Wyatt et al. 2003). As countries become more prosperous, they acquire both the benefits and difficulties of industrialized countries (Popkin, Bing et al. 2002). Nutritionally speaking, the same high-energy, low nutritional value foods that endanger the long-term health of high-income countries, are now widely available in low-income countries. The result is a globalization of nutritional resources where traditional diets, based on unrefined grains and vegetables, have been replaced by meals high in fat and refined carbohydrate, principally as sugars and starch. Food globalization has been facilitated by “improvements” in food production and distribution. For example, subsidized agriculture and multinational companies provide inexpensive refined-carbohydrates and oils (Prentice 2006). Thus, low-priced food allows low-income (few resources) populations increased access to high-energy (but low nutritional value) diets. In this regard, the presence of corporate supermarkets in developing countries increases individual exposure to cheap foods, but since individual food choices in resource-poor populations are based upon price-point and not quality, and high energy processed, poor quality foods are inexpensive, supermarkets provide quick access to foods that are high in fat, sugar and salt, and low in fibre and micronutrients (Popkin 2006). Thus, shifts in diet, from traditional to modern or “Western”, and a wider choice of food sources that encourage people to shift their macronutrient intake pattern, i.e. the nutrition transition as proposed by Popkin (Figure 3).

Urbanization is closely linked to the nutrition transition where towns and cities grow bigger with increased population density and the migration of individuals seeking increased resources, ie. employment. Since 2007, more than half of the world’s population has been living in cities – this is without precedent in human history. The World Health Organization (WHO) projects that by 2050, seven out of ten people will live in urban areas

(<http://www.who.int/mediacentre/factsheets/fs311/en/index.html>). This social phenomenon contributes significantly to the nutrition transition for the following two reasons: first, major dietary changes occur in urban relative to rural areas; second, urban work often demands less physical exertion than rural work (Popkin 2004, Caballero 2005).

Many factors associated with urban life also produce shifts in other areas of lifestyle for people who live in urban areas (Colchero and Bishai 2008, Colchero, Caballero et al. 2008). Urban life implies a particular social organization and environment. Political institutions, industries, and the media play a prominent role in the obesity epidemic through policies, decisions, and product availability (Popkin 2006). Agricultural policies often do not support high-quality, nutritionally beneficial food production, often due to high costs. Although agri-food systems are intimately associated with nutrition, the agriculture and health sectors are largely disconnected in their priorities, policy, and analysis, with neither side considering the complex inter-relation between agri-trade, patterns of food consumption, health, and development (Lock, Smith et al. 2010). Likewise, urban planning often does not provide cities with adequate recreational spaces for physical activity (Colchero and Bishai 2008). With urbanization often comes the processing of food and increased availability of less “healthful” foods to dense populations at specific centres (see supermarkets above). The food industry and media play critical roles in public health and nutrition in the production of both nutritionally rich and nutritionally poor food products and their role in informing the public regarding nutritional components of these products.

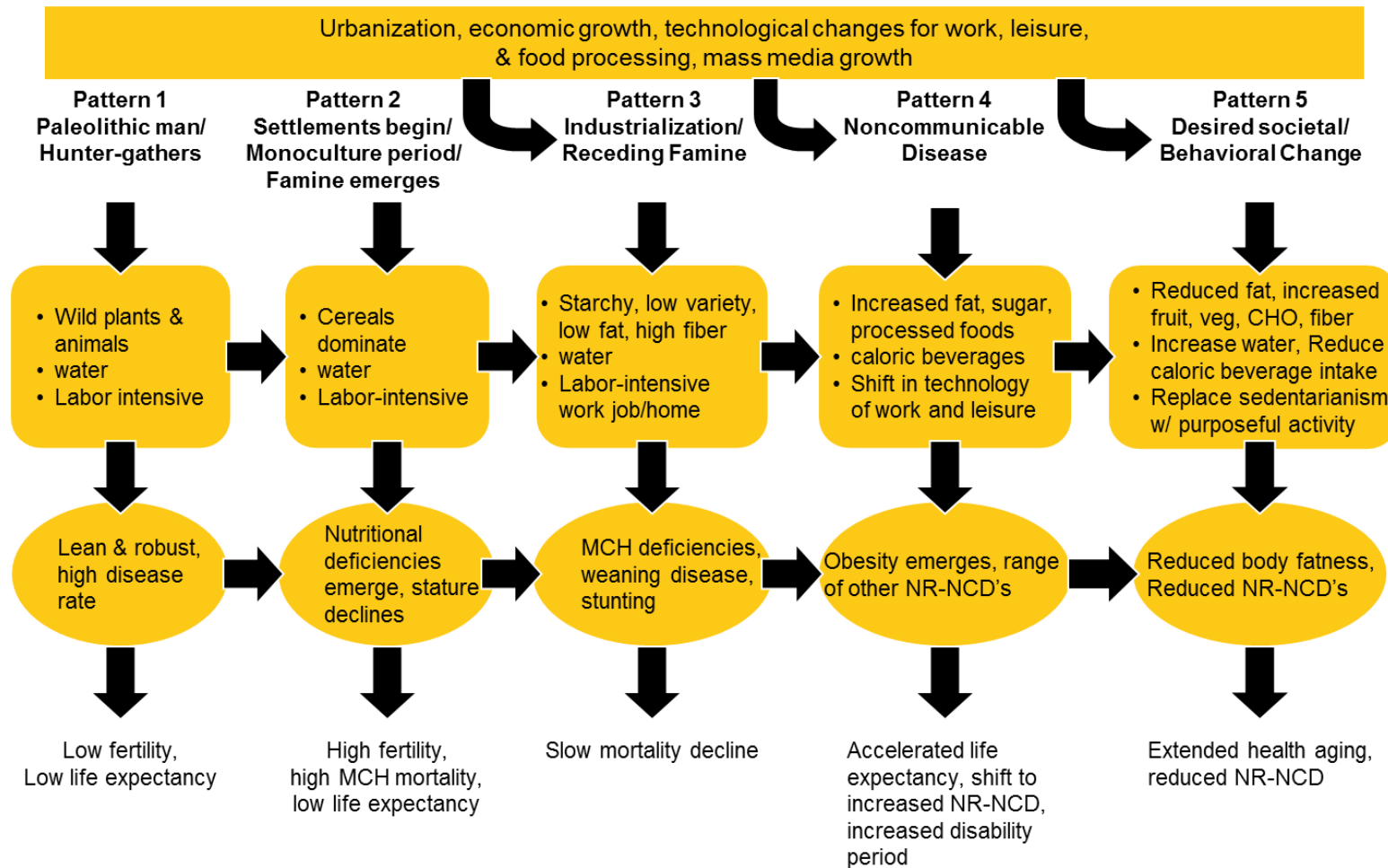


Figure 3. Stages of the nutrition transition

(NR-NCD: Nutrition-Related Non-Communicable Diseases; MCH: Maternal-Child Health). Figure reproduced from Popkin (2002).

Media produces opposite messages, on one hand advertising promote very tempting, tasty, high-calorie food, and on the other promoting thinness as an ideal body shape (Lev-Ran 2001). For example, given all this unhealthy food consumption in the media, how are most of the people in the media so thin and apparently healthy? (Brown and Witherspoon 2002). This paradox –that requires more research because it is unclear- appears to provoke overeating as an immediate gratification (Lev-Ran 2001, Boyce 2007). Thus many a number of social factors influence the obesity epidemic that sweeps both developed and developing countries.

Many social factors affect the amount physical activity in which people engage on a daily basis. For example, industrialization has a major impact on daily physical activity as it increases the use of labour-saving mechanized devices. One can then understand how industrialization may predispose a population to obesity as people change activity patterns (e.g. less activity) while continuing to eat regular amount of calories, creating a positive energy balance (Rennie, Johnson et al. 2005). Socio-behavioural factors, such as motorized transportation, television, computers, and videogames, also reduce physical activity. The school environment - another social factor - often limits the physical activity of the children, especially in developing countries, due to the large number of students per class and inadequate exercise facilities. Another education factor that limits physical activity in schools is that many teachers do not receive training for designing, monitoring, or evaluating programs aimed for a healthy physical activity level (Prentice 2006). In summary, many social changes in developing countries do not promote a healthier lifestyle.

1.3. Dietary Shifts

Changes in the human diet represent the other major component of the nutrition transition. Originally, the Palaeolithic diet consisted largely of high-protein foods and evidence suggests this diet was based on lean meat, fish, vegetables and fruit (Cordain, Eaton et al. 2002). The subsequent adoption of agricultural practices required population-specific adaptations to new environmental pressures, for example, exposure of populations to famine, a variety of local

niches, and the development of social hierarchies. Recently, industrial food processing has altered the nutritional characteristics of foods resulting in an increased glycemic load, increased sodium and fat content, shifts in fatty acid composition, and decreased fibre and vitamins, all of which may contribute to obesity risk (Cordain, Eaton et al. 2005). Consequently, the modern diet has shifted markedly towards an increased intake of energy-dense foods that are high in fat and sugar, but low in complex carbohydrates and fibre (Drewnowski 1997, Popkin and Gordon-Larsen 2004). These dietary changes, based on evidence obtained over the past five decades, clearly suggest that such dietary factors are part of the aetiology of chronic diseases found in many developed parts of the world (Rennie, Johnson et al. 2005).

Hence unhealthy patterns of physical activity and diet, referred to as an “obesogenic environment”, increase the risk of developing chronic, non-communicable diseases. As described above, such environmental factors act from very early in life and not only from direct exposure to the obesogenic environment (Popkin and Gordon-Larsen 2004). Although our understanding of the dietary and molecular mechanisms that promote obesity has advanced over the last three decades, the burden of obesity and its co-morbidities has not diminished. The failure to reduce the prevalence of obesity stems from the fact that excess fat mass is often the result of complex interconnected pathways where factors affecting food consumption play a key role. These factors include food availability, accessibility and choice, which may be influenced by geography, demography, disposable income, urbanization, globalization, marketing, religion, culture, and consumer attitudes. This complexity notwithstanding, a critically important question is how food choices made by humans relates to nutrient intake that, in turn, have a positive or negative impact on human health.

1.3.1. Carbohydrate intake shifts

Dietary changes have followed a similar pattern in a number of different developing countries over the last three decades, changes that consist of a decrease in total carbohydrate intake and an increase in total fat intake. Barbados – an example of a country undergoing the

nutrition transition – experienced increased rates of obesity and chronic non-communicable diseases from 1961 to 2003 while the food supply in 2003 provided over 2500 kilojoules more energy per capita per day than in 1961 (Sheehy and Sharma 2010). During that period, the percent of total daily energy from carbohydrate decreased from 70% in 1961 to 57% in 2003, and the percent of total daily energy from fat increased from 19% to 28% during the same time period. Although total carbohydrate intake decreased, the consumption of simple and complex carbohydrates increased as the intake of complex carbohydrate decreased. For instance, sugar as a percent of total energy increased to over 17%, well above the upper limit set by the WHO (Sheehy and Sharma 2010). These observations serve to support the contention that, in developing countries, the consumption of highly processed foods has increased considerably and such foods are often high in sugar.

The modern diet consists mainly of refined grains and sugar (Popkin 2009). In the nutrition transition, overconsumption of sweetened beverages, especially those sweetened with high fructose corn syrup, exceeds any other dietary change (Bray, Nielsen et al. 2004). Mexico, another country in nutrition transition, consumes more Coca-Cola per capita than the USA (Barquera, Hernandez-Barrera et al. 2008). Using dietary data from 127 countries, Popkin and Nielsen found that caloric sweetener use had increased 74 kilocalories per capita per day globally from 1962 to 2000, with high-fructose beverages as a major contributor (Popkin and Nielsen 2003). A number of hypotheses have been put forward to explain this increase in calories consumed in sweetened beverages. One hypothesis is that humans may lack a physiological basis for processing carbohydrate calories in beverages because only breast milk and water were available for the majority of human evolutionary history (Wolf, Bray et al. 2008). Another hypothesis is that high-carbohydrate beverages may produce incomplete satiation signals which reduce the effectiveness of intake regulation (Wolf, Bray et al. 2008). In this latter argument, fructose differs from glucose in its digestion, absorption and metabolism where hepatic metabolism of fructose favours *de novo* lipogenesis (Khitani and Kim 2013).

More important, fructose does not stimulate an insulin or leptin response that would normally act as key afferent signals to the hypothalamus in the regulation of food intake and satiety. Consistent with this, the overconsumption of sweetened beverages with high-fructose levels increases the total energy intake, raises the risk of obesity (Bray, Nielsen et al. 2004) and intake of sweetened beverages correlates with having an unhealthy diet and obesity (Duffey and Popkin 2006).

1.3.2. Fat intake shifts

Over the last three decades, the consumption of highly processed, high-fat foods has increased in both developed and developing countries (Brooks, Simpson et al. 2010). Not only is daily fat consumption increasing, the ratio of saturated to polyunsaturated fatty acid has also risen (Sheehy and Sharma 2010). Part of this shift is due to economics where the fat intake in modern diet has increased while the relative cost of fat has decreased (Drewnowski 1997). A high-fat diet costs less per unit of energy and may be preferentially selected by low-income consumers and/or those experiencing food insecurity (Drewnowski 1997). This trend is compounded by the fact that many large transnational franchises present aggressive marketing campaigns to penetrate consumer bases. This is particularly relevant in developing countries that may be experiencing increases in disposable income and may be shifting from agricultural based nutrients (traditional grain-based high fibre diets) to processed manufactured foods (Drewnowski 1997).

In general, hunger signals are stronger than satiety signals and although this characteristic was advantageous in times of cyclical energy deprivation, in a modern lifestyle, this contributes to over consumption in an environment that offers a wide range of highly-affordable and palatable (“fast”) foods (Jebb 2005). “Fast” foods are generally high in fat and salt and are a convenient food option in the modern lifestyle due to their accessibility and low cost. Although fast food is not the only cause of the obesity epidemic, it does challenge the human appetite control system as humans select a highly palatable diet when this option is

available. The food industry capitalises on this by increasing the availability of tasty fast-food choices over time (Prentice and Jebb 2003, Mela 2006). More important, in low income families that have few resources and little time to select and prepare meals (because parents work away from home), fast food provides a highly convenient and inexpensive option for eating at home (Adair, Guilkey et al. 2002, Colchero, Caballero et al. 2008). Concurrently, portion size has increased significantly and this, with significant decreases in prices, facilitates excessive consumption and therefore increased total energy intake (Nielsen and Popkin 2003). Thus, fast food represents an important shift in the nutrition transition given its increased consumption is most likely linked to an increased fat, salt, and sugar intake (Prentice and Jebb 2003).

1.3.3. Protein intake shifts

Evidence suggest that specific macronutrients have different effects on appetite and regulation of energy intake (Jebb 2007). While all three macronutrients – fat, carbohydrates and protein - exert some degree of influence on total energy intake, protein is the most satiating and tightly regulated post-absorption (Martens, Lemmens et al. 2013, Gosby, Conigrave et al. 2014). However, up until recently the link between protein and energy intake have been unclear. Whereas carbohydrate and fat intake as drivers of the obesity epidemic have been the focus of the majority of studies, recent theory and data suggest that protein intake may play a predominant role in the regulation of total energy consumption (Simpson and Raubenheimer 2005). Previously, protein has not been linked to the obesity epidemic for the following two reasons: first, protein provides a minority of total energy intake; second, protein intake remains more constant than carbohydrate or fat intake over time and across populations (Stubbs and Elia 2001, Halton and Hu 2004, Stubbs and Lee 2004, Simpson and Raubenheimer 2005).

Simpson and Raubenheimer (2005) used data from the FAOSTAT nutrient-supply database to show that an estimated decrease in dietary protein from 14% to 12.5% between 1961 and 2000 in the USA was associated with a 14% increase in non-protein energy intake, with absolute protein intake remaining almost constant (Simpson and Raubenheimer 2005).

Austin et al. (2011) analysed The National Health and Nutrition Examination Survey showing that a drop in percent dietary protein across the period from 1971 to 2006 has been associated with an increase in total energy intake (Austin, Ogden et al. 2011). Therefore, sufficient evidence supports the potential that protein intake may play a role in total energy intake, but has only received minimal consideration in large population studies, the focus of this thesis.

1.3.4. Summary of the nutrition transition

Major shift in the food environment have occurred over time and resulted in a state of “mismatch”. Humans were physiological adapted to survive in the ancestral environment of limited food availability and evolved owing to the benefits of a hunter-gatherer lifestyle (Gluckman and Hanson 2008). It has been proposed that a critical point occurred when our “ancient” genome collided with new conditions of life, this environmental shift (together the culmination of a nutritional transition with urbanization and commercialization) has supported excess weight gain with unhealthy patterns of diet and physical activity (Popkin and Gordon-Larsen 2004). It has also been proposed that humans are trapped within a physiological “mismatch” as the global lifestyle is changing faster than physiological adaptations to a changing environment through natural selection (Popkin 2006) (Figure 4). The unfortunate health trade-off is manifested as an increased risk for chronic diseases associated with lifestyle shifts including obesity, and Type 2 diabetes (Gluckman and Hanson 2006).

Biology	Technology
Sweet preferences	Cheap caloric sweeteners, food processing benefits
Thirst and hunger/satiety mechanisms not linked	Caloric beverage revolution
Fatty food preference	Edible oil revolution-high yield oilseeds, cheap removal of oils
Desire to eliminate exertion	Technology in all phases of movement/exertion

Figure 4. Mismatch: our biology clashes with modern technology

Figure reproduced from Popkin (2006).

1.4. Human nutritional ecology

A new avenue of nutritional research, developed in the field of nutritional ecology, investigates the manner within which nutrient-specific appetites interact with different nutritional environments to determine food, energy and nutrient intake (Raubenheimer, Simpson et al. 2009). In this context, human nutrition and its health implications are understood as the outcome of regulatory mechanisms that evolved over tens of millennia, operating in environments that are substantially changed from those that provided the selective pressures for the evolution of these mechanisms. The application of this approach in the context of human obesity has proceeded along three fronts, described below.

1.4.1. Experimental studies in animal model systems

Studies in rats, widely used as a model of human nutritional physiology, indicate that these animals regulate their protein and carbohydrates intake. A recent study suggests that protein intake in mice was less well regulated than what has been proposed for humans,

however, tight protein regulation may be responsible for excess energy intake and higher fat deposition when the diet contains a low percent of total energy as protein (Sørensen, Mayntz et al. 2008). A geometric framework has been applied in the study of free-living non-human primates. In the Bolivian rainforest, the spider monkey, *Ateles chamek*, demonstrated nutrient regulatory signals from protein intake (Felton, Felton et al. 2009). A more recent study has shown that mountain gorillas, in their native habitat in Uganda, regulate non-protein energy more strongly than protein, and consequently over-ingest protein when constrained to high-protein diets (Rothman, Raubenheimer et al. 2011), the opposite pattern to that found in mice, spider monkeys and humans.

1.4.2. Experimental studies in humans

Several studies suggest that humans regulate macronutrient intake (Simpson, Batley et al. 2003, Simpson and Raubenheimer 2005, Gosby, Conigrave et al. 2011, Martens, Lemmens et al. 2013, Martens, Tan et al. 2014). Simpson and colleagues applied a multidimensional approach to human nutrition and demonstrated that the traditional focus on energy from carbohydrates and fat, as the proximate cause of obesity, has neglected the important role of protein (Simpson, Batley et al. 2003). Although protein contributes the smallest percentage of energy in the diet, it seems that absolute protein intake remains more stable over time than carbohydrates and fat. An analysis of food consumption patterns from 1961 to 2000 in UK and USA based on FAOSTAT data suggested that, although diet changed, proportional macronutrient intakes remained relatively constant (Simpson and Raubenheimer 2005) (Figure 5). For example, with respect to carbohydrates, a decreased consumption of sugar coincided with an increased intake of complex carbohydrates. With respect to fat intake, decreased consumption of animal fat was associated with increased consumption of vegetable fats. Finally, with respect to protein, increased intake of poultry was associated with decreased intake of beef and pork. Despite this relative constancy in macronutrient proportions, however, total

macronutrient intake increased and the intake of carbohydrates and fat increased at a faster rate than protein intake.

Human nutritional ecology during the Upper Palaeolithic period (35,000 years ago) has been reconstructed by anthropologists and archaeologists. In the Palaeolithic period, the human environment offered limited sources of carbohydrates and abundant dietary protein from the lean mass of animals. During this period, it seems that humans were lean, large and healthy (Simpson and Raubenheimer 1997). There have since been several distinct transitions in the human diet.

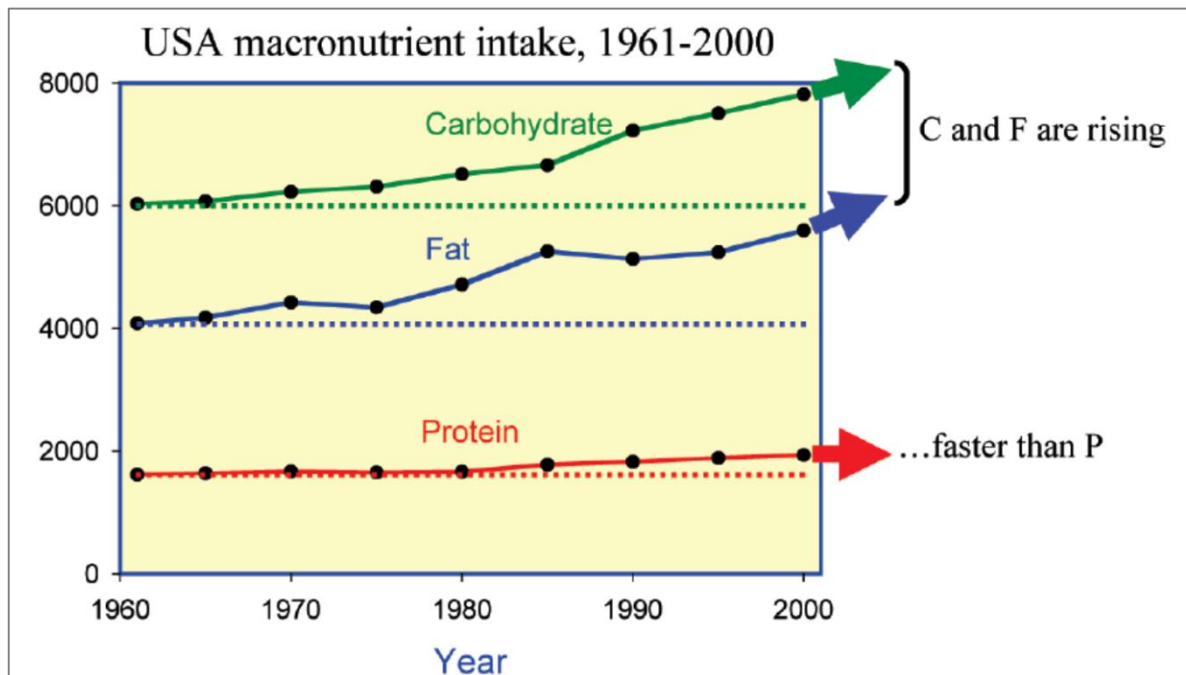


Figure 5. Changing patterns of macronutrient supply in the USA from 1961 to 2000

It is based on FAOSTAT data. Figure and caption reproduced from Simpson and Raubenheimer (2004).

The first transition occurred 10,000 years ago with the development of agriculture, when cultivated grains introduce a large amount of starch into the diet. A second transition occurred

four hundred years ago, during the industrial revolution, when the consumption of sugar increased, people from high socioeconomic groups were more corpulent than people from a low socioeconomic status. With the transition into the modern world, humans have access to all manner of food and macronutrients; humans are large and long-lived, but they suffer from obesity and non-transmissible diseases. In contrast to these large changes in the human nutritional environment, it appears as though our physiology has changed very little (Figure 6).

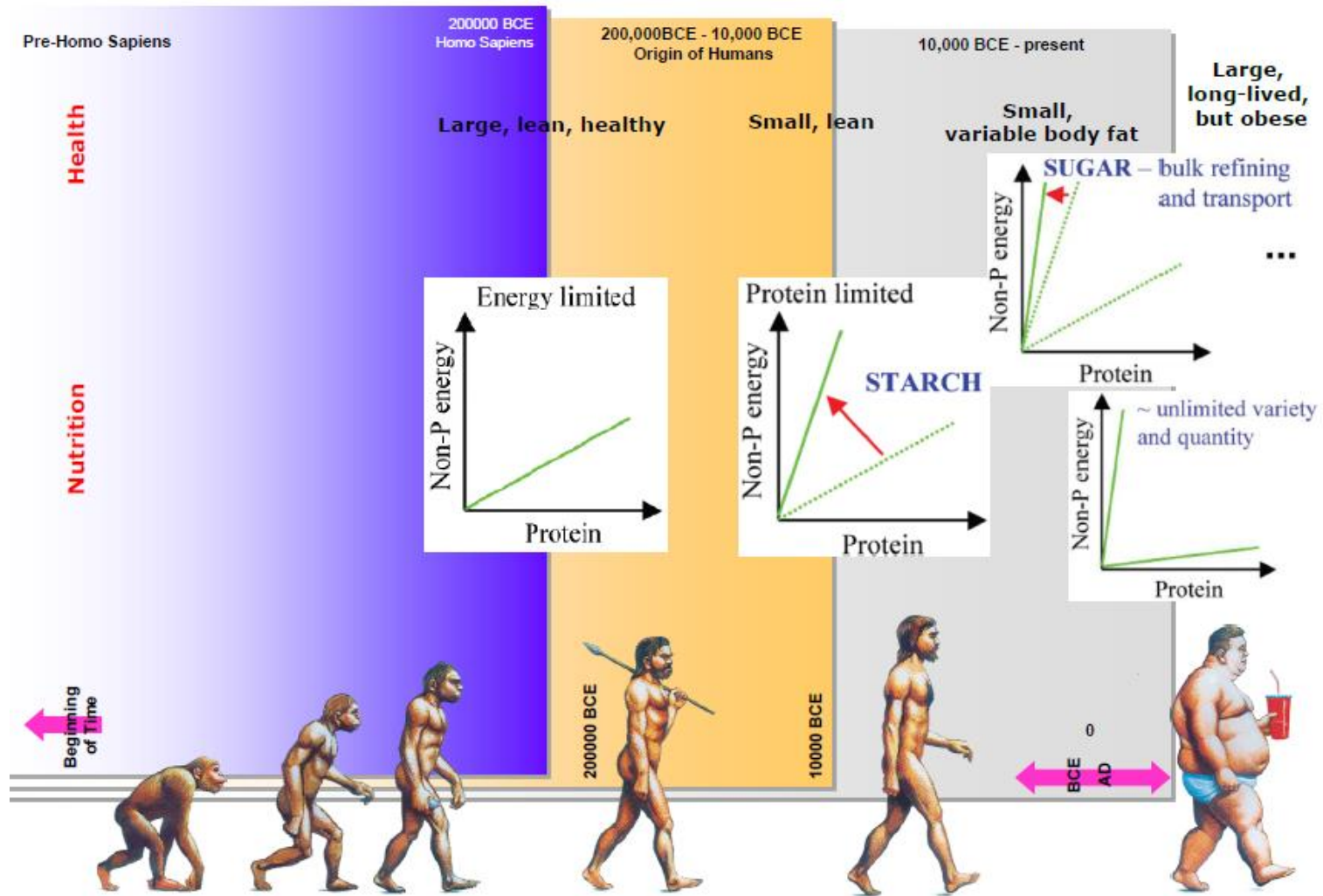


Figure 6. A summary timeline for the changing human nutritional environment since the Paleolithic Era

Figure reproduced from Simpson & Raubenheimer (2004) and Popkin (2008).

1.5. The complexity of human nutrition

Eating, a relatively simple activity, is a challenge to fully understand given the number of factors that influence what a person consumes. Animals innately consume the amount of food they need to eat for survival and growth and make these decisions many times per day throughout their lifetime. Food selection involves a complex decision process. Although humans have evolved a complex brain, it appears that this innate ability to make complex food selection has been challenged and food selection differs among diverse animal species and within the same species at different stages or ages. A key question is, how animals select the kind and amount of food they eat (Simpson and Raubenheimer 1997).

It is well established that obesity results from imbalanced energy consumption relative to energy expenditure, but the role of protein intake regulation in the development of human obesity remains unclear. Intake of particular macronutrients may, theoretically, be regulated and impact on appetite (Jebb 2007). Why people select specific types of foods to eat and how they regulate the quantity consumed remain unanswered questions. New analytical techniques in nutritional ecology have demonstrated that the traditional view of the nutritional environment has some limitations because traditional techniques analyse macronutrient intakes separately. This new vision of nutritional ecology examines the interactions of food components (Raubenheimer and Simpson 1997, Raubenheimer and Simpson 1999) and focuses on protein intake.

1.5.1. The geometry of nutrition

In order to organise ideas and integrate data related to feeding behaviour and nutritional homeostasis, a conceptual framework was developed to provide simple procedures to deal with data on the intake and utilization of macronutrients (Simpson, Batley et al. 2003). This “geometric framework” integrates diet selection, regulation of amount of foods eaten, nutrient

utilization, body composition, and animal performance, a vision that focuses on those aspects of the framework relating to macronutrient regulation.

Simpson and Raubenheimer studied the nutritional biology of animals for more than twenty years (Raubenheimer and Simpson 1997, Simpson and Raubenheimer 1997, Raubenheimer and Simpson 1999, Simpson and Raubenheimer 1999, Simpson, Raubenheimer et al. 2002, Raubenheimer and Simpson 2003, Simpson, Batley et al. 2003, Raubenheimer, Lee et al. 2005, Simpson and Raubenheimer 2005, Simpson and Raubenheimer 2007, Sørensen, Mayntz et al. 2008). In these studies, key aspects of animal nutrition were analysed using a basic geometrical model that considers more than one macronutrient simultaneously and represents a space called the nutrient space, a model that focuses on the interaction among various macronutrients.

The nutrient space

In the geometric framework, an animal is viewed as moving through a multidimensional nutrient space bound by nutrient axes within which exist optimal points or “targets”. The “nutrient target” is the quantity and blend of nutrients provided to the tissues that allow an animal to perform optimally. The “growth target” is the portion of intake optimally used for growth or maintenance of body composition while the “intake target” is the optimal quantity and blend of nutrients ingested with minimal post-ingestive processing costs. The functional “aim” of an animal is to reach these targets, however, when an animal only has access to a single, nutritionally homogenous food, this may not be possible. A food is visualised as a straight line from the origin to the nutrient space and its slope - defined by the ratio of nutrients it contains - is termed a nutritional “rail”. If the nutritional rail passes through the intake target, then the animal can reach the target by eating that food – i.e. the food is nutritionally balanced with respect to the nutrients in the model. By contrast, a rail representing a nutritionally imbalanced food does not lead to the target and does not support a balanced diet. However, the animal could reach its target by combining the intake from two or more nutritionally imbalanced

foods, provided the rails for those foods fall on both sides of the intake target, a process known as “complementary feeding”.

When comparing intake behaviours of groups of animals, each of which has access to a single food varying in nutritional balance (i.e. they are on different nutritional rails), information on macronutrient regulation can be obtained from the shape of the resulting array of intake points. For example, if the intake data cluster more tightly around a single value on one of the nutrient axes, this indicates that the intake of that nutrient is prioritised more strongly than other nutrients in the model. While the framework was developed from studies on insects, it has since been used to study nutrient intake from a variety of taxa, including humans and rats (Raubenheimer and Simpson 1997, Simpson and Raubenheimer 1997, Simpson, Batley et al. 2003). These patterns of nutrient intake reflecting the trade-off between over-eating some nutrients and under-eating others when animals are confined to nutritionally imbalanced foods are known as “rules of compromise” (Figure 7) as follows:

- a) Two-dimensional nutrient space indicating the daily intake of protein and carbohydrates + fat (the ‘intake target’) necessary to maintain energy balance on a diet containing 14% of energy as protein and 86% as carbohydrates + fat, for a moderately active, 45-year-old male (BMI 23.5 kg/m²). A balanced daily diet is the line that runs from the origin to intersect the intake target.
- b) The situation when restricted to an unbalanced diet (one which does not intersect the intake target). If the subject were to eat until point 1, he would have achieved the intake target level of protein but ingested an excess of carbohydrates + fat. At point 2, he would have gained the intake target amount of carbohydrates + fat but under-eaten protein. At point 3, a balance is struck between over-eating the nutrient group in excess and under-eating that in deficit. Here the balance represents the outcome were total energy intake being maintained.

- c) The intake array across five unbalanced diets that describes the rule of compromise in which intake of protein is prioritized over that of carbohydrates + fat.

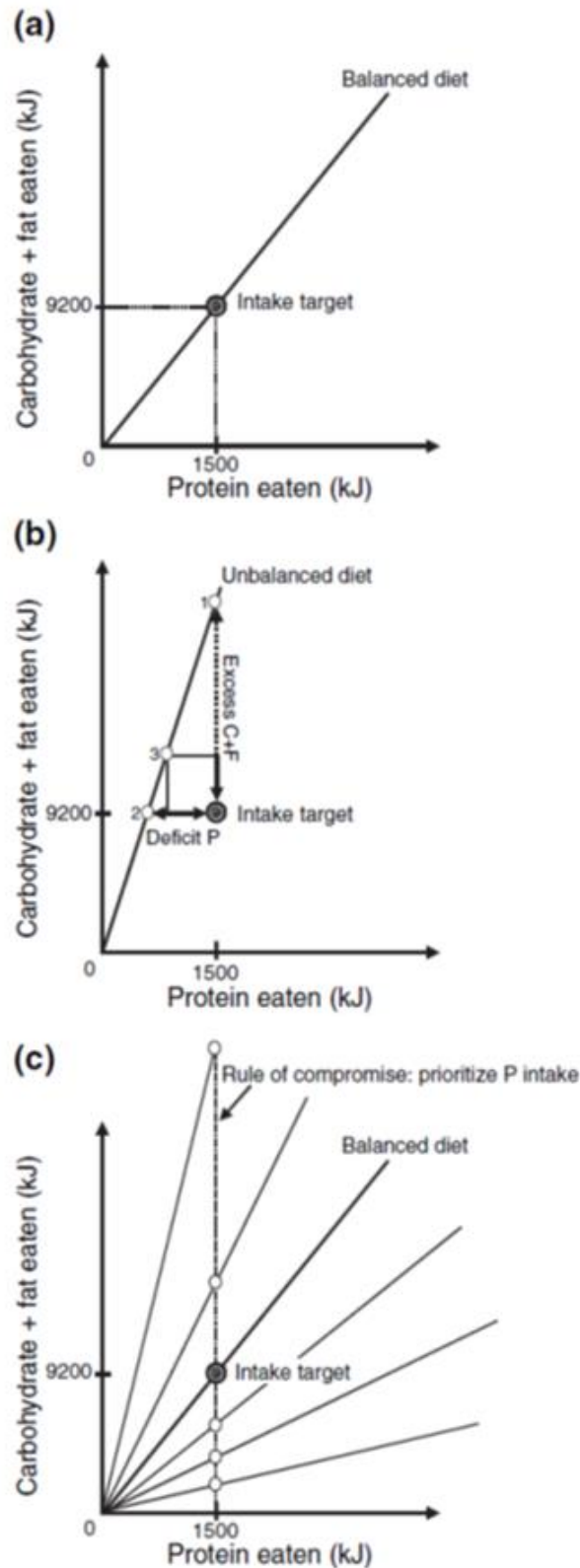


Figure 7. Rules of compromise

Figure reproduced from Simpson and Raubenheimer (2005).

1.5.2. The human rule of compromise

Simpson et al. (2003) used the geometric framework to explore the human rule of compromise for protein and non-protein intake. The experiment involved ten participants (Oxford undergraduate students) who lived together in a Swiss chalet for 6 days. The study included three phases lasting two days each. In Phase 1, the participants were provided with a standard menu consisting of foods spanning a wide range of protein densities; the objective of this phase was to estimate the position of the intake target as the amount and balance of macronutrients selected by the participants with ad-libitum access to nutritionally complementary foods. In Phase 2, two groups of participants were each provided with one of two restricted menus, comprising either food with high or low protein:non-protein energy ratio; the aim of this phase was to estimate the rule of compromise adopted when humans are confined to macronutrient-imbalanced foods. In Phase 3, Phase 1 was repeated to determine whether the participants compensated in their diet selection for the nutritional imbalance accrued in Phase 2 prior period of eating foods either with surplus or deficient treatment diets. In the treatment phase, there were two groups: Group 1 was offered rich-protein foods, and Group 2 was offered low-protein foods. The consumption of foods and macronutrients was analysed and it was observed that over days 5 and 6, the participants who consumed low protein foods ingested less carbohydrates and fat in favour of more protein than the participants who had consumed high protein foods. These results suggest that there is a prioritization of protein (protein intake target).

Four scenarios were developed based in those results (Figure 8):

- i. There is a shift to the diet containing a higher percentage of carbohydrates and fat. If a diet contains low-protein foods (high in carbohydrates and fat), then the consumption of those foods must be higher to obtain the amount of protein required. It may be concluded that a consumption of low-protein foods increases total energy intake.

- ii. There is a shift to the diet containing a higher percentage of protein. If a diet contains high-protein foods, obtaining the protein intake target will result in a lower consumption of carbohydrates and fat, thus less total energy intake.
- iii. There is an increase in the requirement for protein. For example, if a person with a diet of 14% protein increases his demand for protein to 1.5%, this results in a 13% increase in carbohydrates and fat (non-protein intake).
- iv. Diet remains unchanged, but exercise levels decline. If the requirement of non-protein decreases, but the diet composition does not change, maintaining protein intake once again requires overconsumption of carbohydrates and fat (Connolly 2009).

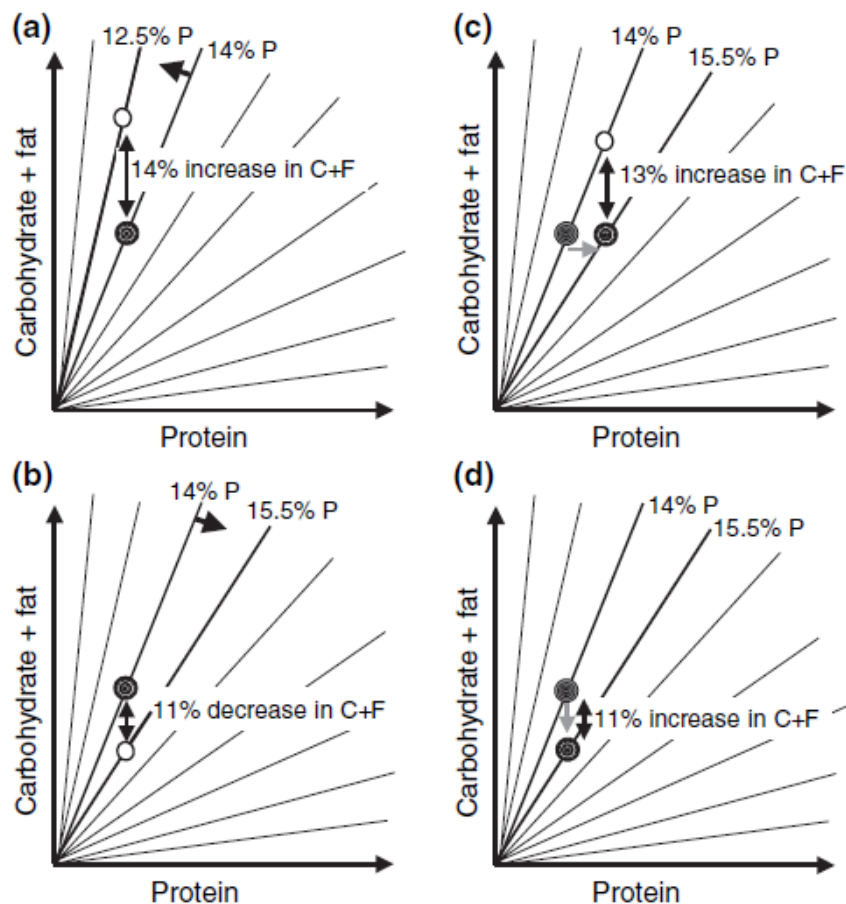


Figure 8. Schematic illustrations of four scenarios in which regulation of protein intake would influence energy balance through its leverage over CHO + FAT intake

(a) The diet changes to containing 1.5% more CHO + FAT (i.e. 1.5% less P). Maintaining P intake requires over-ingesting CHO + FAT by 14%. (b) The diet changes to containing 1.5% more PRO (i.e. 1.5% less CHO + FAT). Maintaining PRO intake is accompanied by 11% reduction in CHO + FAT eaten. (c) The demand for PRO increases, for example, as a result of enhanced rates of hepatic gluconeogenesis as seen in the overweight and obese. If diet composition is maintaining at 14% PRO: 86% CHO + FAT, a 13% increase in requirement for PRO would result in a 13% increase in CHO + FAT intake. (d) The requirement for CHO + FAT is reduced, for example, because of reduced levels of exercise, but diet composition does not change. Maintaining PRO intake once again requires over-consumption of CHO + FAT.

Another important test of the “protein leverage hypothesis” in humans was developed in Australia by Gosby et al (2011) involving 22 lean participants under ad libitum feeding conditions using fixed menus providing 10%, 15% or 25% energy as protein provided in randomly ordered food items designed to be similar in palatability, availability, variety, and sensory quality. This randomised controlled experimental study took place over three 4-day periods in which energy intake and hunger ratings were measured for the participants studied. Total energy intake increased as a result of lowering the percent protein of the diet from 15% to 10%, predominantly from savoury-flavoured foods available between meals. However, the protein leverage was incomplete because the protein intake did not remain constant while the energy intake increased. On the other hand, increasing protein from 15% to 25% did not alter total energy intake. An interesting finding was on the fourth day of the trial during the participants showed a greater increase in the hunger score 1-2 h after the 10% protein breakfast. Therefore, the authors concluded that even when the macronutrient composition of foods was disguised and variety was controlled, increased energy intake occurred on diets containing a lower proportion of energy from protein and persisted throughout the four days of the study. Thus, a change in the nutritional environment that reduces dietary protein relative to carbohydrate and fat intake promotes overconsumption and increases the risk for the potential weight gain (Gosby, Conigrave et al. 2011).

Protein is the most satiating and tightly regulated macronutrient for humans (Stubbs 1995, Stubbs 1998). Some experimental and population-level data studies suggest a similar pattern in which humans tend to prioritize the protein. It is well known that high protein foods are more expensive than low protein foods and may be the reason for the change in dietary patterns in the consumption of protein, especially in developing countries (Brooks, Simpson et al. 2010). Dietary protein in the human diet has had a progressive dilution over recent decades and, at the same time, daily energy intake and the prevalence of obesity have increased.

Therefore, the leverage effect of the protein may play an important role in total energy intake (Simpson and Raubenheimer 2005, Austin, Ogden et al. 2011).

1.6. The “Protein Leverage Hypothesis”

The Protein Leverage Hypothesis (PLH) postulates that humans adjust their food intake to maintain a target protein level (Simpson and Raubenheimer 2005). Subsequently, PLH predicts a negative correlation between protein intake and total energy intake (Simpson and Raubenheimer 1997, Simpson, Batley et al. 2003, Simpson and Raubenheimer 2005, Sørensen, Mayntz et al. 2008). Although protein is a smaller proportion of the human diet (approximately 12-15% of energy) than carbohydrates and fat, protein appears to exert the strongest influence on the regulation of food intake, a small shift in dietary protein content will result in a large change in carbohydrates and fat intake (Simpson and Raubenheimer 1997, Raubenheimer and Simpson 2003, Simpson, Batley et al. 2003, Simpson and Raubenheimer 2005, Weigle, Breen et al. 2005, Sørensen, Mayntz et al. 2008). For example, if a diet reduces protein intake by 1.5%, to compensate for the reduced protein intake requires over-ingestion of carbohydrates and fat by 14% (almost a ten-fold leverage). In other words, PLH states that animals ingest more energy from carbohydrates and fat when they eat low-protein foods (in a low-protein diet) and less energy from carbohydrates and fat when they eat high-protein foods (Simpson, Batley et al. 2003). Thus, animals could ingest less total energy when they eat high-protein foods because the protein target level is attained through a smaller amount of food. Therefore, PLH proposes that protein intake may drive total energy intake and, consequently, determine body weight and may be one of several factors involved in the obesity epidemic (Figure 9).

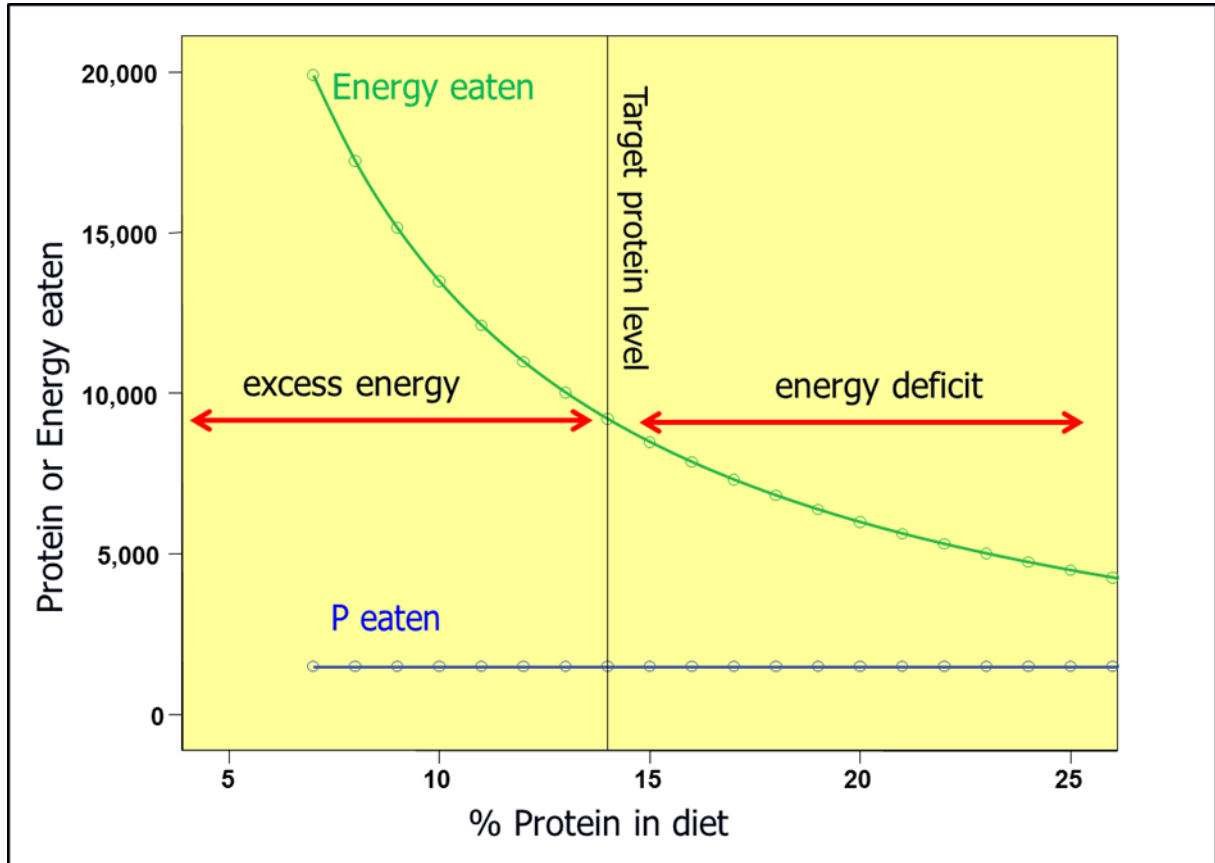


Figure 9. The protein leverage effect

The graph is based on the case where a subject requires a total daily energy intake of 10 700 kJ to remain in energy balance, of which 14% (1500 kJ) is protein. If intake of PRO is strongly regulated, only slight changes in diet composition will have substantial leverage over intake of CHO+FAT and thus energy balance. Because protein comprises only the minor part of the diet relative to CHO+FAT, the effect is especially marked when the shift is to a lower dietary percentage of P, where large excesses of CHO+FAT intake result (diagonal hatched region). A small increase in percentage of PRO in the diet, in contrast, results in under-consumption of CHO+FAT (vertical hatched region). Figure and caption from Simpson and Raubenheimer (2004).

It is important to note that PLH does not make predictions about which foods are eaten, only how much will be eaten for a given dietary composition. Testing PLH in human populations could address an important matter in order to minimize or reverse the obesity epidemic. The leverage effect of the protein may have an important impact on the consumption of carbohydrates and fat, and therefore total energy intake. Developing countries, which have consumed low-protein foods over the last four decades, have a high incidence of obesity. In this

regard, since foods that are higher in protein and lower carbohydrate content are more costly, cheaper food costs and diets high in carbohydrate energy in developing countries, can led to the consumption of excessive energy intake to meet dietary protein needs (Brooks, Simpson et al. 2010).

1.7. Aims and Hypotheses

Although the human diet has changed substantially over time, data suggest that the motivation for making specific food choices is associated with taste, cost and convenience, and likely, with macronutrient targets (Mela 2006). This new vision of nutritional ecology emphasizes the need to measure the interactions of various food components (Raubenheimer and Simpson 1997, Raubenheimer and Simpson 1999). Intake of particular macronutrients may, theoretically, be regulated and have an impact on appetite (Jebb 2007). While all three macronutrients, carbohydrates, fat and protein, exert some degree of influence over total energy intake, protein is the most satiating and tightly regulated macronutrient (Martens, Lemmens et al. 2013, Gosby, Conigrave et al. 2014). Exploring these characteristics of protein, Simpson and Raubenheimer (2005) used a geometric framework based on nutritional ecology to derive the Protein Leverage Hypothesis (PLH). This hypothesis postulates that animals adjust their food intake to maintain a target protein level such that protein intake has a negative correlation with total energy intake (Simpson and Raubenheimer 1997, Simpson, Batley et al. 2003, Simpson and Raubenheimer 2005, Sørensen, Mayntz et al. 2008). Experimental studies and a meta-analysis of macronutrient intake regulation support PLH in both animals and humans, but the hypothesis has yet to be tested in relevant ecological settings (Simpson and Raubenheimer 2005, Gosby, Conigrave et al. 2011). Therefore, the objective of this thesis was to test the PLH in the following two human scenarios: an experimental and free-living human population.

Relevance

High energy-density foods are readily available in the modern diet, but the role of dietary macronutrient composition in the regulation of the amounts of food consumed remains to be determined. The present study provides a picture of patterns of macronutrient intake in the nutrition transition, in which dramatic changes in diet and lifestyle are occurring in countries where urbanization and economy are growing rapidly.

Specific aims

1. To investigate in an experimental setting whether energy intake is inversely proportional to the percentage of dietary protein in Jamaican young adult survivors of severe childhood under-nutrition.
2. To examine the patterns of macronutrient intake in a free-living human population.
3. To identify the association of socioeconomic factors, such as family income and urbanization, with the patterns of macronutrient intake.

Specific hypotheses

I hypothesize that:

1. Small changes in the proportion of protein in the diet modify total energy intake.
2. Regulation of protein intake dominates non-protein intake.
3. Calories derived from protein will remain relatively constant compared to calories derived from carbohydrates or fat in a human population despite changes in family income and urbanicity.

Study one: Testing the PLH in an experimental study

A large experimental study was conducted in Kingston, Jamaica in collaboration with the Tropical Medicine Research Institute at the University of the West Indies. This country was chosen because of the endemic nature of malnutrition among its population. The clinical experiment involved 60 young adult survivors of kwashiorkor and marasmus. These two

distinct syndromes of severe infant undernutrition are characterized by disparate phenotypes, which allowed us a unique opportunity to test PLH. Although I did not design nor implement the entire experimental study, I collaborated in the complete trial and used data to test the PLH in this experimental setting where participants lived in a controlled environment over a 9-day period and ate an unrestricted diet of foods that were manipulated in protein density to test the hypothesis that protein intake dominates over non-protein energy.

Study two: Testing the PLH in a free-living human population

This study used data from the Cebu Longitudinal Health and Nutrition Survey: this survey followed a cohort of individuals over two generations: offspring who were born between 1983 and 1984 ($n = 3,080$) and their mothers. These data are particularly relevant to the investigation of the PLH in relation to the nutrition transition as Cebu is the second largest metropolitan area of the Philippines, and its population is undergoing the nutrition transition. These data were used to test the hypotheses that calories from protein intake remain more constant than those from carbohydrates or fat, even when family income and urbanicity change and that low proportional dietary protein is associated with low socioeconomic status.

Chapter 2

Testing the Protein Leverage Hypothesis in Jamaican adults survivors from severe childhood under-nutrition: randomized experimental study

2.1 Introduction

Although obesity research has focused on carbohydrate and fat intake as the primary main macronutrients involved in the etiology of obesity, recent experimental studies have suggest that protein may be equally, if not more, implicated in the regulation of total energy intake. However, protein intake has not been widely linked to the obesity for two reasons. First, compared with carbohydrates and fat, protein provides a relatively minor part of total energy intake for humans. Second, protein intake remains more constant than carbohydrate or fat intake over time and across populations (Simpson, Batley et al. 2003, Westerterp-Plantenga 2004). The relatively small contribution of protein to total energy intake notwithstanding, among the macronutrients, protein appears to exert the strongest influence on the regulation of intake, leading to the formulation of the Protein Leverage Hypothesis (PLH) for energy intake and obesity (Simpson, Batley et al. 2003, Simpson and Raubenheimer 2005).

The Protein Leverage Hypothesis (PLH) postulates that humans adjust their food intake to maintain a target protein level and consequently will over- or under-eat non-protein energy on low and high-protein diets respectively (Simpson and Raubenheimer 2005). PLH therefore predicts a negative correlation between protein intake and total energy intake (Simpson, Batley et al. 2003, Simpson and Raubenheimer 2005). In other words, PLH states that humans ingest disproportionately more energy from carbohydrates and fat when they eat a low-protein diet and they ingest disproportionately less energy from carbohydrates and fat when they eat a high-

protein diet (Simpson, Batley et al. 2003). This perspective, from nutritional ecology (Raubenheimer and Boggs 2009) emphasises the interaction between human biology (the protein appetite) and the environment (protein density of the diet) that ultimately drives total energy intake and body composition.

Previous experimental studies have supported the PLH – in all cases showing that dietary protein density leverages energy intake - although results have been inconsistent regarding the direction and symmetry of the effect. In an initial study, Simpson et al. (2003) found that protein leverage was symmetrical, yielding both an increase in energy intake on low-protein diet and a decrease on a high-protein diet relative to the self-selected diet. Gosby et al. (2011) found that energy intake was higher on a diet with 10% than 15% protein, but there was no reduction on the 25% diet relative to 15%. Conversely, Martens et al. (2013) found a decrease in energy intake on 30% protein diet relative to 15% but there was no increase on 5% relative to 15% protein. More studies are needed on diverse populations and circumstances to understand this variation in results.

Previous studies were all performed on healthy subjects with no reported severe nutritional deficiencies. Given the considerable evidence that nutrition-related developmental influences can have lasting effects on appetite control (Vickers, Breier et al. 2000, Bellinger, Lilley et al. 2004, Gluckman, Hanson et al. 2005, Gluckman and Hanson 2006), the question arises of whether protein leverage applies more generally to humans with a range of nutritional histories, and if so whether the effect is symmetrical. This chapter presents data from a study that enabled us to test this hypothesis by applying the same techniques employed in the Sydney trial (Gosby, Conigrave et al. 2011) to examine macronutrient regulation in Jamaican young adult survivors from severe childhood under-nutrition.

Aim

The aim of this study was to investigate, in an experimental setting, whether energy intake is inversely proportional to the percentage of dietary protein in Jamaican young adult survivors of severe childhood under-nutrition, and whether any relationship applies both on high and low-protein diets.

Hypothesis

Small changes in the proportion of protein in the diet modify total energy intake.

2.2 Methods

2.2.1 Population

This study was conducted in Jamaica from 2009 to 2011 at the Tropical Medicine Research Institute (TMRI) of the University of the West Indies. Jamaica was chosen because of the endemic nature of malnutrition among its population. This population historically originated from Africa where food insecurity has been prevalent for many generations. The experimental study tested the predictions of the PLH in young adult survivors of severe childhood under-nutrition (SCU). Acute SCU may present as either marasmus (Mr) or kwashiorkor (Kw). Mr is associated with a higher survival rate and wasting while Kw is associated with edema and a poor ability to mobilize substrates such that infants with Kw often die with considerable nutritional reserves (Jahoor, Badaloo et al. 2008).

Jamaica is a low-middle income country with per capita GDP of approximately US\$3,700 (international) and a high GINI coefficient (0.4) reflecting wide social inequalities (Economic Indicators for Jamaica 1990-2003. www.cabinet.gov.jm). Real progress in improving nutrition has been made over the past four decades and SCU, common until the 1980s, is now rare. As the nutrition transition progresses, the prevalence of diabetes, hypertension and obesity have increased (Mendez, Luke et al. 2002).

Sample

An extensive field tracking exercise for survivors of Kw or Mr who had been admitted to the Tropical Metabolism Research Unit (TMRU) Ward for nutritional rehabilitation succeeded in tracking and recruiting 180 individuals, between 19 and 35 years of age. From these individuals, 60 young adult survivors were randomly selected, 29 males and 31 females (Figure 10) with BMI between 18 and 31 kg/m².

Exclusion criteria were pregnancy or planning pregnancy, breastfeeding, known medical conditions or diseases including type 1 or 2 diabetes, kidney, respiratory, gastrointestinal, unstable or untreated elevated blood pressure or cholesterol, cardiovascular disease, or chronic inflammatory conditions. Other exclusion criteria were medications that may interfere with glucose metabolism, smoking, alcohol consumption above current National Health and Medical Research Council guidelines (two standard drinks per day), allergy or intolerance to any of the intervention foods, irregular eating patterns or eating disorder, and following a weight reducing diet. Participants were provided with a complete explanation of the purpose of the study, the nature of any and all risks associated with the measurements, and written informed consent was obtained. The Faculty of Medical Sciences Ethics Committee of the University of the West Indies approved the study.

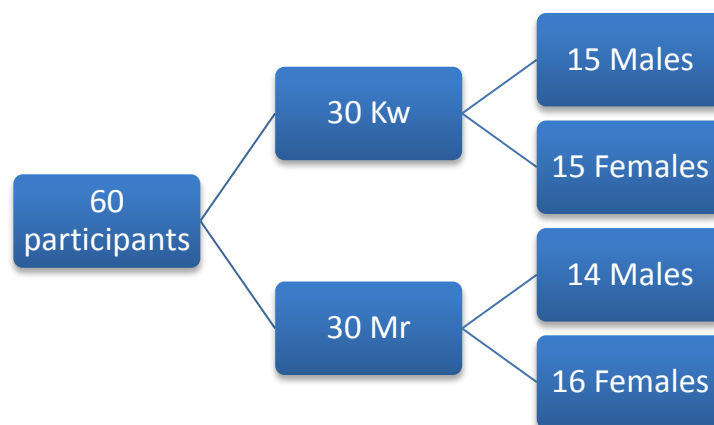


Figure 10. Sample of experimental study

Total number of participants was 60 Jamaican Young adults: 29 males and 31 females.

2.2.2. Diet manipulation

The design, manipulation, and testing of the foods used for the trial was done in collaboration with the University of Sydney (Gosby, Soares-Wynter et al. 2010). Thirty-one local recipes were modified to contain 10, 15 or 25% energy as protein through the addition of food ingredients, a protein mix and/or maltodextrin (Ross Nutrition). Carbohydrate was adjusted to be 60, 55 or 45% energy, and dietary fat was kept constant at 30%. Energy density (kcal/g) was similar among the 10%, 15% and 25% versions of a dish/recipe. Once designed, versions of each food/recipe were taste-tested for pleasantness, as well as the ability of subjects to determine, by taste, the protein concentration of any dish due to appearance, smell or texture. The final ad libitum menus for each day in the 9-day study period contained various combinations of the 31 food items, including 12 sweet and 19 savoury foods (Table 1). Menus were matched for energy density and palatability and up to 11 foods were provided on each day during the 9-day period. Some foods were only available in the meal sittings (breakfast, lunch or dinner) whereas others were snack foods. Snack foods were first served at a meal and could be kept if not eaten or finished at that meal and were labelled for identification and kept in a refrigerator to which participants had free access. All foods were served in weighed quantities on tared utensils, and presented on a single design with white color (Figure 11).

Table 1. Menu

9-day menu was a repetition of 3-day menu, and the percentage of protein varied across the three phases and treatment diets (10%, 15% and 25% protein).

	Study day 1, 4, 7	Study day 2, 5, 8	Study day 3, 6, 9
Breakfast	Cheesy cornmeal muffin	Porridge peanut	Banana bread
	Apple crumble muffin	Banana carrot muffin	Peanut porridge
	Johnny cake	Cheese scones	Savoury muffin
Lunch	Red peas soup	Salsa chilli	Chicken chow m
	Curried potatoes	Cheese pepper cornbread	Potato cheese casserole
	Tuna bake	Mackerel fried rice	Rice & peas
Dinner	Macaroni cheese	Lasagne	Meat loaf
	Seasoned rice	Beanstew	Pelau
	Sweet potato coconut pone	Bread pudding	Sweet potato pudding
Snacks	Orange poppy seed cake	Carrot cake	Bread pudding
	Cornmeal pudding	Coconut custard	Apple crumble cake

**Figure 11. Protein leverage study foods**

The photo shows an example of lunch for two participants. The plates and portions were the same.

2.2.3. Study design

Participants were seen in single-sex pairs and stayed in a dedicated metabolic suite for the entire 9-day period and ate an unrestricted diet of foods manipulated in protein density. Participants arrived for the assigned study period with a completed 3-day food diary. During phase 1 (days 1 to 3) participants ate freely at each mealtime from menus containing food items spanning three protein energy ratios: 10%, 15% or 25% (Gosby, Soares-Wynter et al. 2010). During phase 2 (days 4 to 8), pairs were randomized (each subject was assigned a number; then a table of random numbers was used to select from the numbered participants) to one of three groups each of which received only foods that contained 10%, 15% or 25% protein (Figure 12).

Only the participants were blinded to the treatment. Breakfast was provided at 8 am, lunch at 12:00 pm and dinner at 6 pm. Snacks with macronutrient composition of the assigned trial diet were freely available at all times. Ad libitum energy and macronutrient intake was derived by weighing each food item consumed to the nearest gram using an electronic balance (OHAUS Corporation, Pine Brook New Jersey). A carefully controlled and supervised ‘cumulative weight’ technique was used to record amounts of each food eaten. Each empty plate was used to zero the balance, the first selected food item added to the plate was weighed, the balance re-zeroed and the second-selected food item weighed and so on; left-over food was weighed and subtracted. Macronutrient intake was calculated as the product of the amount eaten and the proportion of each macronutrient within each food type.

Participants were given ad libitum access to study food with no access to other food sources during each experimental period and were supervised during the day. A surveillance system was placed at the external doors of the apartment to monitor compliance during the night. Participants were taken for a 1-hour supervised walk each day at 4 pm. The experimental timeline is shown in Table 2.

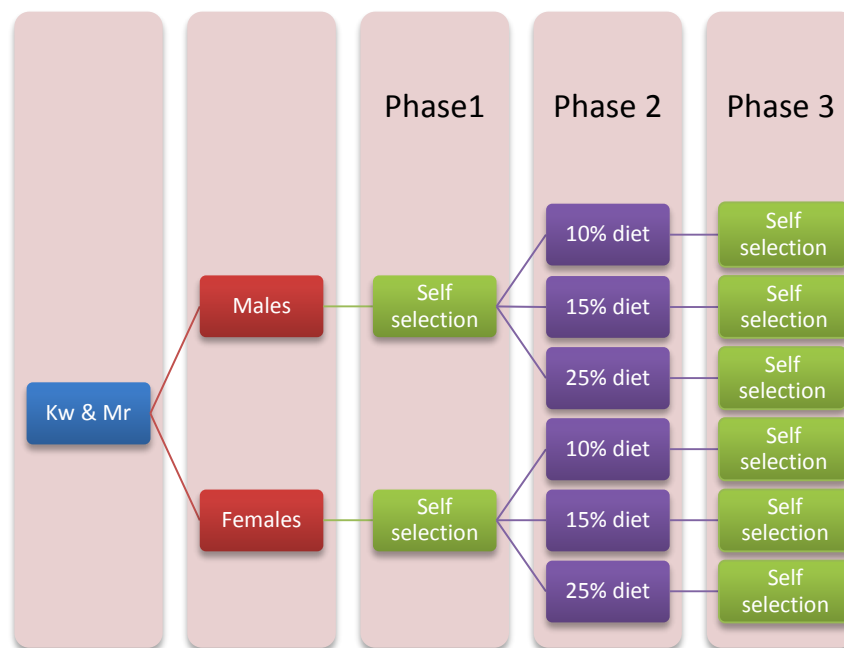


Figure 12. Experiment Phases

All subjects participated in the three phases with phase 2 (experimental phase) differing between treatment groups.

Two nutritional analyses were completed. First, the three-day food diary completed prior to the 9 day ad libitum study period was analysed for daily protein and total energy intake using the NUTRITIONIST Five (version 2.3, 2000, First Data Bank, San Bruno, CA) software. Second, the nutritional basis of food selection was analysed using the Geometric Framework, following protocols have been used previously in humans (Simpson, Batley et al. 2003, Gosby, Conigrave et al. 2011) that allows for the determination of satiety regulation by protein, fat and carbohydrate. There are three phases to the experiment, during each of which the participants were provided a variety of foods that varied in macronutrient ratios, with these phases differing in the range of macronutrient ratios being offered.

Table 2. Experiment timeline

Experiment 9-day period in Jamaica	
4-day pre-admission (Thu)	Make contact with participants Obtain consent Give instruction for 3-day food diaries (Fri, Sat, Sun). Obtain information on food allergies/intolerance
Admission day (Mon)	Participants arrive at the study apartment (Medical history was done previously)
15:00	
17:00	Dinner
Day 1,2&3	
Phase A: Standard Diet	
7:00	At the study apartment the food is reheated and weighed for breakfast.
7:30	Height Body weight
8:00-9:00	Breakfast
9:00	Left-over food is weighed and subtracted from initial weights to derive an accurate measure of intake.
9:30-12:00	Participants engage in structured activity-skills training (computer courses), games, etc.
10:30-11:30	Food for lunch is prepared; every portion of food is weighed/measured
11:30	Food is provided ad libitum in excess and participants invited to eat lunch
12:00-13:00	Lunch
13:00	Left-over food is weighed and subtracted from initial weights to derive an accurate measure of intake
13:30	Snacks foods were labelled for identification and stored in a refrigerator, to which the participants had free access at all times.
14:00-16:00	Participants engage in structured activity-skills training (computer courses), games, etc.
16:00	Supervised walk (College Common-see route specified)
17:00	Return to study apartment
17:30	Food for dinner is prepared; every portion of food is weighed/measured
18:00	Food is provided ad libitum in excess and participants invited to eat dinner
18:00-19:00	Dinner
19:00	Left-over food is weighed and subtracted from initial weights to derive an accurate measure of intake
Day 4,5,6,7&8	

Phase B:**K10%, K15%, K25% Protein foods****M10%, M15%, M25% Protein foods**

7:00	Researcher arrives at the onsite kitchen to collect foods for the day's meal. At the study apartment the food is prepared for breakfast, every portion of food and drink is measured. Leftover food (snacks from the previous evening) is weighed and subtracted from initial weight to derive an accurate measure of individual intake. Body weight
8:00	Breakfast
9:00	Left-over food is weighed and subtracted from initial weights to derive an accurate measure of intake
9:30-12:00	Participants engage in structured activity-skills training (computer courses), games, etc.
10:30-11:30	Food for lunch is prepared; every portion of food is weighed/measured
11:30	Food is provided ad libitum in excess and participants invited to eat lunch
12:00-13:00	Lunch
13:00	Left-over food is weighed and subtracted from initial weights to derive an accurate measure of intake
13:30	Snacks foods were labeled for identification and stored in a refrigerator, to which the participants had free access at all times.
14:00-16:00	Participants engage in structured activity-skills training (computer courses), games, etc.
16:00	Supervised walk (College Common)
17:00	Return to study apartment, leftover food from snacks weighed and subtracted from last weights
17:30	Food for dinner is prepared; every portion of food is weighed/measured
18:00	Food is provided ad libitum in excess and participants invited to eat dinner
18:00-19:00	Dinner
19:00	Left-over food is weighed and subtracted from initial weights to derive an accurate measure of intake.

Day 9**Phase C: Standard Diet**

7:00	Researcher arrives at the onsite kitchen to collect foods for the day's meal. At the study apartment the food is prepared for breakfast, every portion of food and drink is measured. Leftover food (snacks from the previous evening) is weighed and subtracted from initial weight to derive an accurate measure of individual intake. Body weight
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8:00	Breakfast
9:00	Left-over food is weighed and subtracted from initial weights to derive an accurate measure of intake
9:30-12:00	Participants engage in structured activity-skills training (computer courses), games, etc.
10:30-11:30	Food for lunch is prepared; every portion of food is weighed/measured
11:30	Food is provided ad libitum in excess and participants invited to eat lunch
12:00-13:00	Lunch
13:00	Left-over food is weighed and subtracted from initial weights to derive an accurate measure of intake
13:30	Snacks foods were labeled for identification and stored in a refrigerator, to which the participants had free access at all times.
14:00-16:00	Participants engage in structured activity-skills training (computer courses), games, etc.
16:00	Supervised walk (College Common)
17:00	Return to study apartment, leftover food from snacks weighed and subtracted from last weights
17:30	Food for dinner is prepared; every portion of food is weighed/measured
18:00	Food is provided ad libitum in excess and participants invited to eat dinner
18:00-19:00	Dinner
19:00	Left-over food is weighed and subtracted from initial weights to derive an accurate measure of intake.
Day 10	
Last Measurements	
7:00	Researcher arrives at study apartment to collect the participants to go to TMRI
7:30	Body weight
8:00	Breakfast (normal diet)
9:00	Participants leave study apartment

Phase 1. Self-selection: day 1, 2 & 3. Participants were provided with a variety of foods spanning a wide range in their balance of protein:carbohydrate and allowed to freely select their meals. The aim of this phase was to assess the ad-libitum selected macronutrient intakes of the participants.

Phase 2. Treatment: day 4, 5, 6, 7& 8. Each group of participants was randomly split into three sub-groups, each of which received menus of 10, 15 or 25% protein. The menus

differed only in the ratio of protein:carbohydrate and were otherwise similar. The aim of this phase was to compare the responses of the participants to diets that were mildly imbalanced with either an excess or deficit of protein vs. carbohydrate. Specifically, measures of macronutrient intake in these circumstances provided an indication of the extent to which the participants over-ate one macronutrient (e.g. protein in the high-protein treatment) in order to ingest the target level of the other (e.g. carbohydrate). Such measures provide a powerful indicator of the relative priorities that the regulatory systems of consumers assign to different macronutrients.

Day 9. All participants were returned to the same wide-ranging menu offered in the first phase.

2.2.4. Statistical analysis

Statistical power was based on detecting differences for the a priori hypothesis between two independent means of total energy intake between 10% protein diet vs. 25% protein diet. To detect a 2% difference in total energy intake between the two groups with a 95% power (α 0.05) it was estimated that nine participants per groups would be required. The difference in total energy intake was based on previous research (Gosby, Conigrave et al. 2011).

Statistical analysis was performed using SPSS Version 21 (IBM Corporation, New York, USA). All variables were tested for normal distribution using the Kolmogorove-Smirnov, ShapiroWilk tests and normality plots. Data are reported as mean (95% CI) from summary statistics.

As body composition is not the same for all participants (e.g. fat free body mass differ between males and females), energy intake was calculated as a percentage of energy requirements (energy requirements were calculated based upon the Harris & Benedict equation, multiplied by the estimated physical activity level ≈ 1.6). In the treatment diet (phase two),

gender was used as an interaction factor in order to test the possibility that male and female respond differently to the different protein diets.

Nutrient intake data were analysed using ANOVA to determine differences between the 10%, 15% and 25% protein diet. When significant effects were observed, post-hoc analysis with Bonferroni adjustments were performed to determine which time points differed significantly. A P-value of <0.05 was considered statistically significant.

2.3. Results

2.3.1. Sample

Sixty adult survivors of severe childhood under-nutrition aged 19 to 35 years were studied. All participants had been treated at the Tropical Metabolism Research Unit (TMRU) during childhood. Gender, age, and severe childhood under-nutrition did not vary significantly among the protein diet groups. BMI differed between 10% and 25% protein groups (Table 3), but this was not statistically significant at a Bonferroni-corrected significance level of 0.0166 ($0.05/3$ comparisons), nor were there significant differences in any other pairwise comparison. Overall, 18 participants were underweight, 23 were normal-weight, 13 were overweight, and six were obese. Habitual energy and macronutrient intake from the 3-day food diaries are summarized in Table 4. Males consumed an average of 14.7% of total energy from dietary protein and females consumed an average 15.1% of total energy from dietary protein. One subject was omitted because he ate very little compared with mean of intakes in phase two and, for consistency, this subject was also omitted from the analyses of phases one and three.

Table 3. General characteristics for the participants by 10%, 15% and 25% protein diet

	10%	15%	25%	Df	F-value	P-value
Gender (M/F)	10/11	10/10	9/10	2		0.98 ^a
Age (year)	27.8±7.3 ^b	25.3±6.0	26.8±7.4	2	0.6	0.51 ^c
BMI (kg/m²)	25.2±4.8 ^b	22.2±6.0	21.2±3.1	2	3.6	0.03 ^c

M, male; F, female; BMI, body mass index; ; ^a Chi-Square test was used to determine differences between the 10%, 15% and 25% protein; ^b Values are means ± SD; ^c ANOVA was used to determine differences between the 10%, 15% and 25% protein. Post-hoc analysis was performed using Bonferroni, deriving a significance level of 0.0166 (=0.05/3 comparisons).

Table 4. Reported habitual energy and macronutrient intake prior to the study (3-day food diaries)

Kcal	Severe acute malnutrition phenotype			
	kwashiorkor		marasmus	
	male n=12	female n=12	male n=12	female n=13
Energy (kcal)	1731.6±459.5	1737.6±824.8	2219.5±1133	1999.3±990.9
Protein (kcal)	253.3±66.5	240±120.2	338.2±187	322.1±162.9
Carbohydrate (kcal)	1113.6±343.6	1138.8±498.9	1357.3±697	1082.1±422.4
Fat (kcal)	372.3±5.4	371.4±239.4	534±318.6	591.6±493.2
Protein (%)	14.7±1.8	13.9±4.0	14.8±2.8	16.3±3.2

Values are means ± SD.

*Only 49 participants completed the food diaries.

2.3.2. Phase one: self-selection

The aim of Phase 1 (self-selection) was to estimate a mean protein target. Although there were no diet groups in this phase, the statistical analysis was made according to assigned diet group for phase 2 (Table 5). Since BMI differed between groups, energy requirements differed, so energy intakes were calculated as a percentage of energy requirements. ANOVA test determined no differences in energy intake (as a percentage of energy requirements), protein, carbohydrates, or fat intakes as percents of total energy intake between the three protein-diet groups. The mean protein intake for all participants was 402 (114) kcal and the

percent of total energy intake as protein did not differ between males and females (14.6% males and 14.84% females).

If participants were to have eaten randomly among the 10%, 15% and 25% protein foods provided during self-selection phase, they would have consumed 16.7% of total energy as protein during that phase. The results obtained were lower than expected as the observed percent of total energy intake as protein was 14.6 %, 14.8 %, and 14.8 %, for the 10%, 15% and 25% protein-diet groups, respectively. However, the participants were close to metabolic equilibrium as estimated habitual protein intakes were similar to protein intake during the choice experiment. Habitual percent dietary protein intake was estimated to be approximately 14.7 (2.3) % and 15.1 (3.6) % for males and females, respectively.

Table 5. Energy and nutrient intakes on Phase 1 (Self-selection)

	10%	15%	25%	df	F-value	P-value
	N=10/11	N=10/10	N=9/10			
Energy intake as percentage of energy requirement	118.3±30.8	107.0±32.6	100.6±27.0	2,57	1.67	0.197
Protein (kcal)	438.0±129.4	388.8±139.3	361.8±119.2	2,57	1.78	0.177
Non-protein (kcal)	2574.4±771.1	2215.0±707.6	2102.1±691.9	2,57	2.21	0.119
Fat (kcal)	867.4±258.4	748.3±254.5	703.1±238.9	2,57	2.31	0.108
Carbohydrate (kcal)	1703.0±476.8	1466.6±453.6	1527.9±472.8	2,57	2.42	0.097

Values are means \pm SD. ANOVA was used to determine differences between the 10%, 15% and 25% protein.

2.3.3. Phase two: treatment diet

Phase two was analysed independent of phase one rather than the difference in intake between phase one (self-selection) and phase two (leverage). The reason for this is that Phases 1 and 2 of the experiment differed in several respects over-and-above the experimental manipulation (macronutrient selection vs. no-choice, respectively) that were not possible to control, and between-phase comparisons were thus considered not reliable. For example the

initial novelty for subjects of being provided with free access to diverse foods in phase one would no longer apply in phase two; likewise, subjects entered phase 2 having spent the prior three days eating experimental diets, whereas prior to phase 1 they had eaten their usual diets. By contrast, within phase 2 all three treatment groups were subjected to the same conditions over the preceding three days, and the within-phase comparison was thus valid.

There was no significant gender x diet interaction term, and therefore sexes were combined in reporting diet effects (Table 6). Energy intake as percentage of energy requirement of 25% protein diet was lower compared to the 10% diet group as shown in Table 6. Participants consumed 807.7 Kcal more non-protein energy on a 10% than 25% protein diet (P10% vs. 25%: $P < 0.0001$).

Table 6. Energy and nutrient intakes in phase 2 (leverage) of the 10%, 15% and 25% protein diet

	10% N=10/11	15% N=10/10	25% N=9/10	df	F-value	P-value
Energy intake as percentage of energy requirement	116.2±28.3	104.7±24.7	87.1±29.2†	2,57	5.6	0.006
Protein (kcal)	297.4±87.2	366.4±102.3	502.2±215.6†‡	2,57	10.2	0.01
Non-protein (kcal)	2451.1±1108.1	2178.2±567.9	1643.4±668.7†	2,57	4.9	<0.0001
Fat (kcal)	855.7±252.4	730.5±204.0	606.7±261.1†	2,57	5.3	0.007
Carbohydrate (kcal)	1820.6±507.5	1447.7±364.5†	1036.6±407.9†‡	2,57	16.3	<0.0001

Values are means ± SD. ANOVA was used to determine differences between the 10%, 15% and 25% protein. Post-hoc analysis was performed using Bonferroni.

† compared to 10%;

‡ compared to 15%.

2.4. Discussion

Results from this experimental study provide further evidence that dilution of the protein content of the human diet can result in energy over-consumption, as proposed by the PLH. This study tested the pattern of macronutrient regulation in a population of subjects with a history of

severe under-nutrition living in a country undergoing the nutrition transition. The general pattern of macronutrient regulation was similar to that previously found in subjects in the UK (Simpson and Raubenheimer 2005) and consistent with results from Australia (Gosby, Conigrave et al. 2011) and The Netherlands (Martens, Lemmens et al. 2013). In the present study, total energy intake increased on diets containing 10% relative to 25% protein diet suggesting protein leverage as demonstrated by Simpson et al. (2003).

Whereas previous experimental studies of PLH have involved subjects with a history of nutritional sufficiency (Simpson and Raubenheimer 2005, Gosby, Conigrave et al. 2011, Martens, Lemmens et al. 2013), this study was performed on subjects from a very different culture and with a history of severe nutritional deficiencies in childhood, a factor that, according to the developing origins theory, may influence development of food preference (Bellinger, Lilley et al. 2004). Additionally, the fetal programming of these participants could contribute to adult hyperphagia (Vickers, Breier et al. 2000, Gluckman, Hanson et al. 2005). Using the same techniques employed in the Sydney trial, macronutrient composition of foods was disguised and variety was controlled (Gosby, Conigrave et al. 2011), it was found that protein leverage applies also to experimental subjects with a history of severe childhood undernutrition.

The physiological mechanisms of the human protein appetite are as yet unclear, but significant advances have recently been made (Simpson, Le Couteur et al. 2015). Griffioen-Roose et al. (2014) used functional magnetic resonance imaging (fMRI) to show that human brain reward responses to savoury food cues are specifically heightened in mildly protein-deprived compared with protein-satiated humans. This suggests that dietary protein status alters taste-category responses, a mechanism which the authors conclude is likely to play an important role in the regulation of protein intake in humans. The nutrient signalling systems controlling protein appetite are thought to involve both lean hormonal signals and circulating amino acids (Simpson, Le Couteur et al. 2015). Laeger et al. (2014) showed that hepatic fibroblast growth factor 21 (FGF21) production, which has been linked to metabolic adaptation to starvation in

humans, is induced specifically by dietary protein restriction, and not energy restriction. The authors conclude that FGF21 is an endocrine signal specifically of protein restriction. Both protein seeking and FGF21-associated protein appetite are likely to play a role in the protein leverage effect.

In the self-selection phase (phase one), the balance between protein and non-protein intake did not differ between protein diet groups, with all groups self-selecting. Phase two (leverage) was analysed independent of self-selection phase (target) and showed that energy intake increased on diets containing a lower proportion of energy from protein. In other words, the participants ingested more energy from carbohydrates and fat when eating a low-protein diet compared with a high-protein diet. That this progressive dilution of protein in the diet composition differed significantly from random suggests that it is the result of homeostatic regulation of macronutrient intake – i.e. an intake target. An interesting question is how general this target of 15% protein is to humans. Given the wide range of protein intakes observed across human populations – from approximately 10% in the Okinawan Japanese, to 35% in traditional Inuit (Raubenheimer, Rothman et al. 2014, Raubenheimer, Machovsky-Capuska et al. 2015), it seems likely that the target level of protein is adaptable. One factor that has been proposed to influence the target protein level is the density of dietary protein in the diet during early development (Raubenheimer, Rothman et al. 2014). Specifically, exposure to high dietary protein levels in early development, as is the case for formula-fed human infants, is hypothesised to condition reduced protein efficiency and increase the protein target. This is significant point as the strength of protein leverage is increased in proportion to the value of the protein coordinate of the intake target (Simpson and Raubenheimer 2005, Raubenheimer, Rothman et al. 2014). Phase two (leverage), which was analysed independent of self-selection phase (target), showed that a decrease in dietary protein density (10% relative to the selected 15%) may be a trigger for increased energy intake. This finding has potentially important implications from a public health perspective, simply that factors that might result in dilution

of the protein content of the diets of Jamaicans might lead to excess energy intake and obesity. One factor that has been highlighted by Brooks et al. (2010) is economics given that protein is more expensive than other energy sources and, consequently, there might be economic incentives by producers and/or consumers of foods to dilute the protein content and may account for the progressive dilution of the percent of total energy from protein in the diets of some countries over the last four decades with associated rises in energy intake and obesity, including the USA (Simpson and Raubenheimer 2005, Austin, Ogden et al. 2011). From these results it is plausible that participants may increase their body weight if they continue to consume increased energy as found when they consumed a diet low in protein.

The present study and Gosby et al (2011) aimed to test PLH in a randomized, controlled experimental study by using a variety of foods and food choices in which protein and carbohydrate were exchanged and energy density and palatability kept similar. The relative protein content of the diet: 10% of energy, 15% of energy and 25% of energy from protein was similar in both studies. Differences in the design between the present study and that of Gosby et al (2011) were the number of subjects ($n = 60$ compared with $n = 22$), characteristics of the subjects (previously malnourished compared to normally nourished individuals) and duration of the experiments (1 time for 9 d compared with 3 times for 4 d). The present study demonstrated a significant increase in energy intake when participants consumed a low-protein diet. Gosby et al showed that participants had a higher energy intake when consuming a low-protein diet (10% of energy from protein) compared with a normal protein diet (15% of energy from protein) and no difference in energy intake compared to a high-protein diet (25% of energy from protein). The prevention of increased energy intake with a high protein diet is in accordance with Stock's hypothesis (Stock 1999), which postulates that energy efficiency is influenced by dietary protein content. Consistent with both the present study, Simpson et al. (2003), Gosby et al. (2011), and Martens et al. (2013) reported that energy intake decreased on a high (30%) compared with an intermediate (15%) protein diet. However, in contrast with all

other studies, Martens et al. did not find an increase in energy intake on the low, compared with intermediate- protein diet. A potentially important difference in the design of Martens et al. is that the low protein diet contained only 5% protein, whereas all studies that have found increased energy intake with protein dilution, including the present study, have used a low protein content of 10%. As discussed by Raubenheimer et al. (2014), 5% protein is lower than the dietary protein level of any human society with protein sufficiency, and considerably lower than the USA (10%) and Australia/New Zealand (15%) minimal AMDR level, being approximately equal to the protein level of potatoes or white bread. It is possible that the subjects in the study of Martens et al. could not sustain an intake of such an extreme diet over the 12-day experimental period, possibly even developing learned aversions to the extreme diet. Thus, it would be of interest to re-test that population comparing energy intakes on diets of 15% vs. 10% protein.

Our data are consistent with results of Gosby et al. (2013), who performed a meta-analysis of 38 published experimental trials comparing energy intakes of subjects eating diets of different protein content. The results showed that energy intake increased across a range of dietary protein densities spanning 8-54%. Raubenheimer et al. (2015) have extended this analysis to include 22 additional data points that were not available at the time of publication of Gosby et al.'s meta analysis, including the results of Martens et al. (2013). Again, a statistically significant negative relationship between dietary protein density and energy intake was found, despite the inclusion of Marten et al.'s 5% protein treatment. In summary, there is consistent evidence that dietary protein content leverages energy intake, although further work is needed to understand the circumstances under which the directionality and symmetry of leverage varies.

Our study is not without limitations, however, and we recognize that our 3-day food diaries included 2 weekend days, which may have over estimated macronutrient intake (Ma, Olendzki et al. 2009).

In conclusion, we found evidence to support bidirectional protein leverage as subjects consumed more energy on a diet containing a lower percent of protein compared to a diet with a higher percent of protein and lower energy intake on a high compared to a low protein diet. Evidence of protein leverage in Jamaican subjects with a history of severe under-nutrition as well as in UK, Dutch, and Australian subjects suggests that this phenomenon is widespread and very well may be universal among humans. This result does not in itself demonstrate that protein leverage has a significant role in the obesity epidemic, but it does highlight a potential for protein dilution to interact with human regulatory physiology to increase global obesity. A logical next step to testing this hypothesis is to collect evidence of protein leverage in free-living humans, the subject of the next chapter.

Chapter 3

Testing the Protein Leverage Hypothesis in a free-living human population

3.1. Introduction

The nutrition transition refers to the dramatic changes in diet and lifestyle in low and middle income countries where economic development and urbanization are proceeding rapidly (Colin Bell, Adair et al. 2002, Eckhardt, Adair et al. 2003, Bray and Popkin 2007). Despite the increased prevalence of obesity globally, how individuals regulate the quantity and types of food items they consume remains only partially understood. Some studies indicate that food choices are associated with taste, cost and convenience (Mela 2006, Brooks, Simpson et al. 2010). In addition, there is growing evidence that specific macronutrients have different impacts on appetite and the regulation of energy intake (Jebb 2007). While all three macronutrients –carbohydrates, fat and protein– exert some degree of influence on total energy intake, protein is the most satiating and tightly regulated (Halton and Hu 2004, Weigle, Breen et al. 2005, Soenen and Westerterp-Plantenga 2008, Gosby, Conigrave et al. 2011, Griffioen-Roose, Mars et al. 2011).

Simpson and Raubenheimer developed the Protein Leverage Hypothesis (PLH) which postulates that, as a consequence of relatively strong protein appetite compared with the other two macronutrients, humans adjust their food intake to maintain a relatively constant dietary protein intake and, are more likely to have higher energy intakes on diets with low protein density (Simpson, Batley et al. 2003, Simpson and Raubenheimer 2005). There is growing support for PLH from experimental studies and meta-analyses of macronutrient intake regulation (Simpson, Batley et al. 2003, Simpson and Raubenheimer 2005, Gosby, Conigrave et al. 2011, Gosby, Conigrave et al. 2014).

Despite the studies discussed above, PLH has yet to be tested outside of experimental settings in free-living humans. In human nutrition, a wide range of factors affect food consumption: availability, accessibility and choice, which may be influenced by geography, demography, disposable income, urbanization, globalization, marketing, religion, culture, consumer attitudes, etc. (Popkin 2004). Evidence also suggests that specific macronutrients may have different impacts on appetite and regulation of energy intake (Jebb 2007). The leverage effect of the protein could have a huge impact in the consumption of carbohydrates and fat, and therefore in the total energy intake. Higher food prices are associated with higher protein content and lower carbohydrate content; therefore, diets high in carbohydrate energy can lead to consume excessive energy to meet the dietary protein needs (Brooks, Simpson et al. 2010). In this chapter, we presented results from a study that tested central predictions of PLH in the face of dietary changes in free-living human populations.

Specific aims

1. To examine the patterns of macronutrient intake in a free-living human population.
2. To identify the association of socioeconomic factors, such as family income and urbanization, with the patterns of macronutrient intake.

Specific Hypotheses

1. The regulation of protein intake dominates non-protein intake.
2. Calories from protein intake remain more constant than those from carbohydrates or fat in a human population even when family income and urbanization change.

3.2 Methods

3.2.1 Population

Data studies were collected in the Cebu Longitudinal Health and Nutrition Survey (CLHNS) that began in 1983 and follows a cohort of women residing in Metropolitan Cebu

City in the central Philippines (Adair, Popkin et al. 2011). Cebu is one of the fastest growing and most rapidly developing regions of the Philippines and shares many characteristics with other large cities in transitional Asian countries. For instance, the mean real household income in Cebu nearly doubled among study participants from the 1980s to 1990s and was accompanied by shifts in diet and physical activity patterns (Colchero, Caballero et al. 2008). The CLHNS is a community-based cohort study of a city with 1.5 million inhabitants. The 33 local communities randomly selected for the survey included densely populated urban neighborhoods, peri-urban neighborhoods, and surrounding rural villages. All pregnant women in these communities were invited to participate and were included in the study if they gave birth between May 1983 and April 1984. The final sample contained 3,080 single live births. The cohort of children who were born during that period, their mothers, other caretakers, and selected siblings had been followed through subsequent surveys conducted in 1991-2, 1994, 1999, 2002 and 2005. A central theme in CLHNS is linking the effects of social factors and biomedical factors on morbidity, growth, and mortality (<http://www.cpc.unc.edu/projects/cebu/>). The CLHNS has been reviewed and approved by the University of North Carolina Institutional Review Board, Office of Human Research (Adair, Gultiano et al. 2011).

3.2.2 Sample

This study used longitudinal CLHNS data to test the associations of within-population factors, such as family income and urbanicity, on protein intake over time. The longitudinal study was restricted to mothers for each survey year. The offspring were not included because they were too young to have a substantial impact on their choices independent of maternal preferences. This sample included repeated measurements for these women in 1986, 1994, 1998, 2002, and 2005; the 1983 survey was excluded because all women who participated in the CLHNS were pregnant. Table 7 presents the characteristics of the sample for this study.

Table 7. Characteristics of Cebu Longitudinal Health and Nutrition Survey (CLHNS) women across survey years

Survey	1986	1994	1998	2002	2005
<i>N</i>	2031	1880	1831	1656	1380
Age, <i>y</i>	28.5 (20.7-40.5)	37.8 (30-50.1)	42 (34-53.8)	44.9 (37.4-57.2)	48 (40-59.6)
BMI, <i>kg/m</i>²	20.1 (16.7-25.8)	22.8 (17.6-30.2)	23.3 (17.2-30.7)	24.3 (17.6-31.6)	24.3 (17.7-31.9)
Energy Intake, <i>kcal</i>	1205.9 (494.2-2546.7)	1283.9 (563.5-2436.8)	1278.9 (568-2433.6)	1171 (503.2-2479.8)	1088.3 (485.6-2118.2)
Protein intake, <i>kcal</i>	141.4 (57.7-335)	164.0 (68.6-363.2)	160 (64-366.4)	163.7 (58-394.2)	158 (60.2-363.6)
Protein intake, %	11.7(4.7-27.7)	12.7(5.3-28.1)	12.5(5-28.6)	13.9(4.9-33.4)	14.5(5.5-33.3)
Carbohydrates intake, <i>kcal</i>	900.8 (394.2-1800.1)	889.6 (418.7-1622.3)	857.5 (422.2-1600.6)	790.7 (363.9-1481.3)	741.3 (356.2-1312.1)
Carbohydrates intake, %	74.6(32.6-89)	69.2(32.5-86.1)	67(32.9-85)	67.5(31-82.4)	68.1(32.7-83)
Fat intake, <i>kcal</i>	89.8 (15.1-649.4)	148.9 (23-853.2)	163.5 (25-865.7)	131.8 (19.1-887.7)	137.8 (20.5-648.7)
Fat intake, %	7.4(1.2-53.5)	11.5(1.7-65.8)	12.7(1.9-67.2)	11.2(1.6-75.4)	12.6(1.8-59.3)
Household income, <i>pesos</i>	164.4 (34.2-635.9)	381.8 (114.4-1266)	425.9 (157.8-1320.8)	443.4 (150.3-1555.1)	440.1 (139.4-1672.2)
Urbanicity index	30 (8-47)	39 (12-51)	41 (14-56)	45 (16-59)	43 (16-57)

Values are medians (5th-95th percentile)

3.2.3 Instruments

We used data from 24-hour dietary recalls to examine whether population-level data are consistent with PLH. One day of intake was recorded at each sampling time by highly trained local field staff. Data were collected during in-home interviews and staff at the University of North Carolina converted food consumption values into kilocalories per day using food composition tables from the Philippines (Kelles and Adair 2009).

3.2.4 Data analysis

Variables

At each sampling time, dietary intake was measured by trained interviewers using 24-hour recall, and the reported intakes were used in the analyses. Calories of dietary protein (PRO), carbohydrates (CHO), and fat (FAT) were calculated by multiplying grams intake by 4 (calories per gram) for PRO and CHO, and by 9 for FAT.

Household income was derived from the sum of all household members' cash-income and the value of in-kind earnings. For compatibility over time, income values were deflated using year-appropriate Philippines consumer price indices. Urbanization was derived from an urbanicity index score based on the following seven criteria: population size, population density, communication, transportation, healthcare services, education, and market availability; assets was derived from an index score based on the following criteria: electricity, own house, material, air conditioner, own TV, own car, tape record, and refrigerator (Dahly and Adair 2007, Colchero, Caballero et al. 2008, Victora, Adair et al. 2008).

Longitudinal analysis

These longitudinal data were used to test the hypothesis that protein intake remains more constant than fat or carbohydrates over time. Data from 2,031 24-hour dietary recalls from women in 1986, 1994, 1998, 2002, and 2005 were analysed. Additionally, the relationship

between macronutrient intake and socioeconomic factors, such as household income and urbanization, was tested in this population undergoing shifts in diet and lifestyle.

Longitudinal analyses were used to characterize the changes in macronutrient intakes during the 20-year period (from 1986 to 2005). All analyses were performed using SAS V9.2. To investigate differences in the change in energy intake from PRO, CHO and FAT over time, a general linear mixed model was fitted to the data with time and macronutrient (PRO, CHO or FAT) as repeated measures. A spatial power covariance structure was fitted to the measurements within a subject over time. Different variance estimates for each macronutrient were used and subject was included as a random variable. Age at time 1, macronutrient, time (measured in years) and the macronutrient-time interaction were included as explanatory variables. The interaction, testing the difference in slopes over time for the different macronutrients, was the test statistic of interest. As the distribution of energy intake was skewed, the log of energy intake was used as the dependent variable.

To investigate whether household income and urbanization influenced the difference for change in macronutrient intake over time, general linear mixed models were fitted to the data with both time and macronutrient (PRO, FAT or CHO) as repeated measures. A spatial power covariance structure was fitted to the measurements within a subject over time, with different variance estimates for each macronutrient and subject included as a random variable. Age at time 1, macronutrient, time (measured in years) and household income or urbanicity index, and the 2 and 3 way interactions of time and macronutrient with household income or urbanicity index were included as explanatory variables with the 3 way interaction testing whether the difference in slopes over time for the different macronutrients differed depending on household income or urbanicity index. As the distribution of calorie intake was highly right skewed, the log of calorie intake was used as the dependent variable. Where the 3 way interaction was found to be significant random coefficients general linear mixed models were fitted separately for calories from PRO, FAT and CHO. Time was a repeated measure with

individual subject intercepts and slopes across time being modeled as random effects. Age at time 1, macronutrient, time (measured in years), household income, urbanicity index, and the interactions of time with income and urbanicity index were included as explanatory variables. Initially, income and urbanicity index were included as continuous variables, but in order to investigate the interactions they were categorized: urbanicity index into six equally spaced levels, and household income into 12 equally spaced levels (tail categories grouped because of small numbers). As the distribution of energy intake was skewed, the log of energy intake was used as the dependent variable.

3.3. Results

3.3.1. Longitudinal study

Macronutrient intake, household income and urbanicity index varied over time. The slopes of the relationship between time and caloric intake from each macronutrient differed significantly ($p < 0.0001$). PRO kcal intake increased over time [slope (SE) = 0.005 (0.0009)], but at a slower rate than FAT kcal intake [slope (SE) = 0.02 (0.002)] while CHO kcal intake decreased slightly [slope (SE) = -0.008 (0.0007)] as shown in Table 8.

Table 8. The estimates of the slopes

Nut	Estimate	Standard Error	Pr > t
CHO	-0.008	0.0007	< 0.0001
FAT	0.019	0.0018	< 0.0001
PRO	0.005	0.0009	< 0.0001

There was strong evidence for differences in the rate of change in macronutrient intake over time by income and urbanicity (both $p < 0.0001$), therefore, the macronutrients were analysed separately. In relation to PRO, there was evidence for change in PRO intake over time

depending on income ($p = 0.003$) and urbanicity ($p = 0.006$) (Tables 9&10). Estimates of the slopes of change in PRO intake over time were negative and more negative as income or urbanicity level increased. Also, there was evidence of an effect of both urbanicity ($p < 0.0001$) and income ($p = 0.02$) on change in CHO intake over time, again with the slope becoming more steeply negative as income or urbanicity level increased (Tables 11&12). Finally, there was strong evidence for an effect of both urbanicity and income (both $p < 0.0001$) on the change in FAT intake over time, again with the slope becoming more steeply negative as urbanicity or income level increased (Tables 13&14). In summary, there was a decrease in PRO Kcal intake over time as household income increased or index of urbanicity increased. However, the effect on PRO was very small, and that on both FAT and CHO more marked (Figure 13).

Table 9. The association between income and the change in protein intake over time

Income level	Slope	Standard error	P value
Income1	-0.00382	0.001640	0.0199
Income2	-0.00714	0.001556	< 0.0001
Income3	-0.00752	0.002238	0.0008
Income4	-0.00826	0.003304	0.0125
Income5	-0.00303	0.004836	0.5316
Income6	0.003833	0.006139	0.5324
Income7	-0.00599	0.008738	0.4932
Income8	-0.00759	0.01020	0.4571
Income9	-0.01891	0.01190	0.1121
Income10	-0.01903	0.01339	0.1554
Income11	-0.00228	0.01023	0.8238
Income12	-0.01289	0.01144	0.2601

Table 10. The association between urbanicity and the change in protein intake over time

Urbanicity level	Slope	Standard error	P value
Urbanicity1	-0.00540	0.004326	0.2120
Urbanicity2	-0.00659	0.002279	0.0038
Urbanicity3	-0.00399	0.002492	0.1092
Urbanicity4	-0.00567	0.001942	0.0035
Urbanicity5	-0.00841	0.001709	<0.0001
Urbanicity6	-0.00717	0.002831	0.0113

Table 11. The association between income and the change in carbohydrate intake over time

Income level	Slope	Standard error	P value
Income1	-0.01198	0.001347	< 0.0001
Income2	-0.01548	0.001300	< 0.0001
Income3	-0.01536	0.001848	< 0.0001
Income4	-0.01493	0.002713	< 0.0001
Income5	-0.01105	0.003937	0.0050
Income6	-0.01515	0.004990	0.0024
Income7	-0.01900	0.007094	0.0074
Income8	-0.01681	0.008355	0.0443
Income9	-0.00792	0.009665	0.4126
Income10	-0.01465	0.01078	0.1742
Income11	-0.03120	0.008347	0.0002
Income12	-0.00845	0.009441	0.3708

Table 12. The association between urbanicity and the change in carbohydrate intake over time

Urbanicity level	Slope	Standard error	P value
Urbanicity1	-0.00990	0.003420	0.0038
Urbanicity2	-0.01117	0.001802	<.0001
Urbanicity3	-0.01433	0.002026	<.0001
Urbanicity4	-0.01173	0.001585	<.0001
Urbanicity5	-0.01558	0.001398	<.0001
Urbanicity6	-0.02143	0.002177	<.0001

Table 13. The association between income and the change in fat intake over time

Income level	Slope	Standard error	P value
Income1	0.007079	0.003241	0.0290
Income2	-0.01519	0.003078	< 0.0001
Income3	-0.01073	0.004419	0.0152
Income4	-0.02246	0.006520	0.0006
Income5	-0.01966	0.009537	0.0393
Income6	-0.02658	0.01210	0.0281
Income7	-0.03541	0.01722	0.0398
Income8	-0.03883	0.02012	0.0536
Income9	-0.03206	0.02345	0.1717
Income10	-0.03261	0.02637	0.2161
Income11	-0.01854	0.02018	0.3583
Income12	-0.04580	0.02258	0.0426

Table 14. The association between urbanicity and the change in fat intake over time

Urbanicity level	Slope	Standard error	P value
Urbanicity1	0.004550	0.008122	0.5753
Urbanicity2	-0.00348	0.004287	0.4174
Urbanicity3	-0.00161	0.004821	0.7391
Urbanicity4	-0.00713	0.003749	0.0574
Urbanicity5	-0.01034	0.003301	0.0017
Urbanicity6	-0.03247	0.005359	< 0.0001

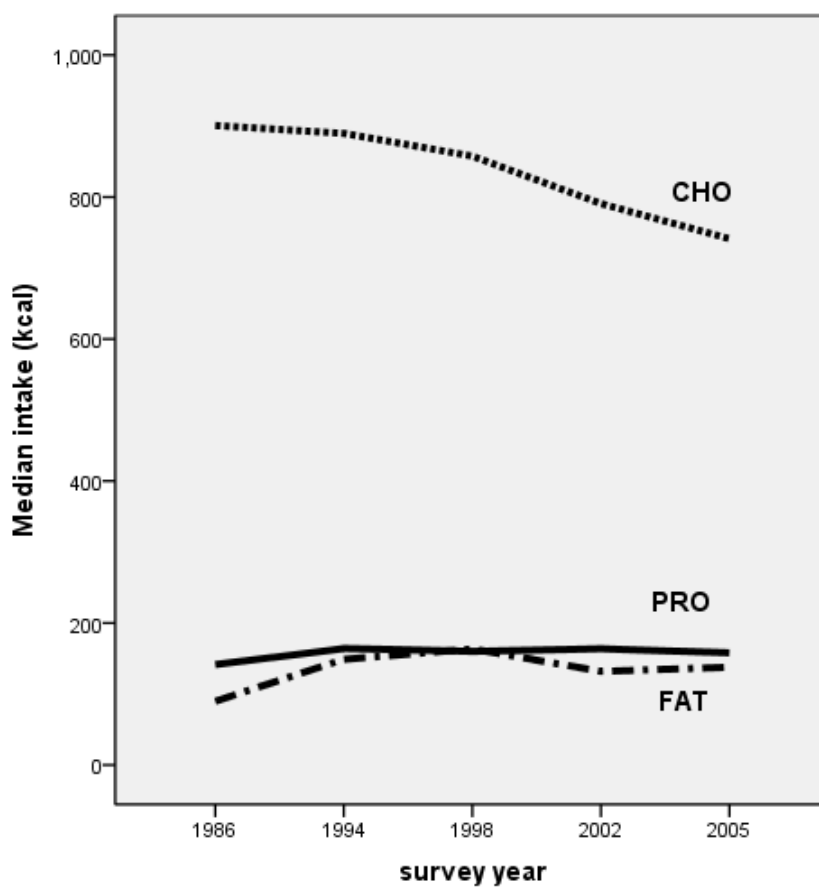


Figure 13. Macronutrient intakes of Cebu Longitudinal Health and Nutrition Survey (CLHNS) women across survey years

The slopes of the relationship between time and log of kcal intake from each macronutrient differed significantly ($p < 0.0001$). PRO intake increased slightly over time [slope (SE) = 0.005 (0.0009)], but at a slower rate than FAT intake [slope (SE) = 0.02 (0.002)] while CHO intake decreased slightly [slope (SE) = -0.008 (0.0007)].

3.4. Discussion

In this longitudinal study population, macronutrient intake changed during the decades of observation. It was hypothesized that protein intake would be most conserved as these changes unfolded. In support of this prediction, our analyses showed that caloric from dietary protein remained more constant over time than caloric intake of dietary carbohydrates or fat, even when data were controlled for the absolute level of each macronutrient in the diet. Although calories from dietary protein increased slightly over time, the increase was at a slower rate than that for fat, while carbohydrates decreased slightly. These findings are consistent with the interpretation that the total amount of protein consumed is more tightly regulated than intake of carbohydrates or fat (Simpson, Batley et al. 2003, Simpson and Raubenheimer 2005, Popkin 2006, Sheehy and Sharma 2010). The results are also consistent with trends in the USA, where absolute protein intake increased from the 1970s to the 2005-2006, but at a slower rate than the intake of non-protein energy increased, and consequently the density of protein in the diet decreased (Austin, Ogden et al. 2011). Over this period there was significant increase in the incidence of obesity, as predicted by the PLH (Raubenheimer, Rothman et al. 2014). As discussed in Chapter 1, the observed trends are also consistent with the general pattern in the nutrition transition, in which energy intake increases while cheap carbohydrates and oils displace protein from the diet (Popkin 2009).

The increase in protein intake observed over the study period, albeit considerably smaller than the decrease in carbohydrates, may reflect an interaction between regulatory biology and socioeconomic changes that affect the relative affordability of different macronutrients (Brooks, Simpson et al. 2010). The dietary shift found is consistent with patterns observed in other low or middle income countries (Popkin 2006, Popkin 2009). The socioeconomic structure of the study population changed over time with some female participants moving into higher income and urbanicity categories over the course of the study.

Despite the fact that calories of dietary fat and protein increased slightly over time, calorie intake decreased among women who remained in the same income and urbanicity category. As urbanicity or income scores increased over time, the change in macronutrients intake became more steeply negative: the macronutrient intake of women in the lowest income or urbanicity category showed the smallest decrease while women in the higher income or urbanicity categories showed the highest decrease.

Although previous studies using the CLHNS data have found minimal evidence of selection bias due to attrition of subjects during the course of the study, such problems are worth bearing in mind (Adair, Gultiano et al. 2011). Another possible limitation of this study is that dietary recall systematically underestimates energy intake (Goris and Westerterp 2008); furthermore, one 24-hour diet recall were used when three 24-hour recalls are viewed as more reliable and appear optimal for estimating energy intake (Ma, Olendzki et al. 2009). It should be stated that the energy intake s are not observed, but reported energy intakes.

Balancing these potential limitations, the strengths of this study lie in the detailed environmental, socioeconomic, and demographic information of the CLHNS from individual, household and community-levels that provides an opportunity to explore how changes in patterns of macronutrient intake relate to changing community and household level conditions over a 20-years period. More longitudinal studies need to be conducted to explore dietary shifts in developing countries where the obesity epidemic and its related chronic diseases are the most serious challenge to long-term health.

In conclusion, our longitudinal findings indicate that energy from protein intake remained more constant than that from carbohydrates or fat in a human population undergoing shifts in diet and lifestyle over a two-decade period, even in different income and urbanization categories. These findings are consistent with the idea that recent changes in the protein density of the human diet have played a causal role in the developing obesity epidemic – the Protein Leverage Hypothesis (Simpson and Raubenheimer 2005). Further work is needed to examine

the generalizability of this effect and, in particular, to identify and account for exceptions – namely, cases where dietary protein density decreases with time, but there is no accompanying increase in energy intake, or cases where energy intake increases concurrent with increasing proportional dietary protein content. Above all, research is needed to understand the relative role of protein leverage in temporal, geographic and socioeconomic variation in energy intake and obesity, and how protein leverage interacts with other factors. Obesity is a complex problem and it is only through taking a broad view of the causes that this problem can be understood and managed.

Chapter 4

General Discussion

Human obesity has increased rapidly over the past three decades. Over the same period of time, human biology has changed minimally, suggesting that environmental changes has contributed significantly to the rise in obesity. Macronutrients are clearly implicated in the obesity epidemic, but there is uncertainty as to their relative roles. Many studies of obesity have focused on carbohydrates and fat, but the Protein Leverage Hypothesis (PLH) suggests that protein might play a key role. Experimental studies show evidence of a relationship between protein intake and appetite regulation (Weigle, Breen et al. 2005). In 2003, a UK study (Simpson, Batley et al. 2003) reported that humans over-eat energy on low-protein diets and under-eat energy on high-protein diets, as predicted by PLH. However, the Swiss study did not control food palatability, participants might have eaten more of the low-protein foods because they tasted better. A subsequent study, in Sydney, was designed to control for palatability by standardizing diets with 10%, 15% and 25% protein (Gosby, Conigrave et al. 2011). Despite the comparable palatability, participants on the low-protein (high-carbohydrate) diet over-ate energy. In 2013, Martens et al. found decreased energy intake on high protein diets as predicted by PLH. The Australian, UK and Dutch studies together provide strong evidence for protein leverage in humans, but are limited in two respects: the subset of the human population was very limited, and experimental settings do not represent the realistic environment in which health outcomes are determined.

The aims of this thesis were, first, to extend the study of protein leverage to two additional populations, and second, to extend the study to a new context, namely diet surveys of human intakes outside of the experimental setting. The first test of protein leverage was in humans with a history of severe childhood under-nutrition. Given the growing body of work

showing the importance of developmental history in predicting energy intake and obesity (Vickers, Breier et al. 2000, Gluckman and Hanson 2004, Gluckman and Hanson 2006, Gluckman, Hanson et al. 2007, Gluckman and Hanson 2008), if that population showed protein leverage as did the populations from UK, Sydney and the Netherlands, this would be a particularly interesting test of the generality of PLH. The second test of protein leverage, was performed using population data estimating intakes in free-living humans. The Cebu Longitudinal Health and Nutrition Survey provided a sensitive test of the PLH because the Filipino population surveyed is undergoing the nutrition transition, which is associated with shift in diet and nutrient intake.

4.1. Experimental study

Chapter two describes the test of protein leverage in humans with a history of severe childhood under-nutrition. Results suggested that the participants showed protein leverage as did the participants in the UK and Sydney study (Simpson, Batley et al. 2003, Gosby, Conigrave et al. 2011). This test was particularly interesting for the generalizability of PLH given the theory predicting that nutritional history should impact on energy balance and health outcomes by a mismatch pathway. This pathway includes the evolved adapted responses of the developing organism to anticipated future adverse environments which leads to maladaptive consequences if the environment is mismatched to that predicted (Gluckman and Hanson 2004, Gluckman, Hanson et al. 2007, Gluckman and Hanson 2008). There is some experimental evidence to suggest that this does happen. For example, Vickers et al. (2000) showed in studies of rats that when offspring of undernourished dams are cross-fostered to normally-nourished dams at birth a mismatch is generated, in which the ensuing catch-up growth of the offspring results in a greater risk of chronic disease as adults (Vickers, Breier et al. 2000). This mismatch pathway might perturb the appetite control mechanism creating the conditions for the later pathological effects of an obesogenic diet (Gluckman, Hanson et al. 2008), or perturb developmental

programming that can lead to alterations in food choice behavior in offspring (Bellinger, Lilley et al. 2004).

The experimental study of subjects undernourished during childhood in Jamaica consisted of two kinds of diets: standard, in which participants could self-select the protein content of their diet, and treatment in which they were confined to one of three menus with a fixed proportion of energy from protein. The macronutrient composition of foods was disguised and variety controlled in both diets. Gosby et al. (2010) had developed a selection of foods with differing proportions of protein but equal palatability in two settings: Sydney Australia and Kingston Jamaica. The foods were manipulated to contain 10, 15 or 25% protein with carbohydrate to 60, 55 or 45% respectively, and dietary fat was kept constant at 30%. The important achievement in the design of these diets was that the tasting panel participants did not identify the difference in protein content between these foods, and the versions were rated equal in pleasantness.

In the self-selection diet, the balance of protein to non-protein energy self-selected did not differ between groups. The foods, including both sweets and savory options, were offered to participants under ad-libitum feeding conditions. These foods had different protein content, and the participants could select any of them and any amount of food. Consuming more sweet or more savory foods could have resulted in different macronutrient intakes; however, participants consumed similar macronutrient proportions. This suggests that the participants regulate their intake to a protein:non-protein energy macronutrient target.

In the treatment diet, we tested whether the participants over-ate energy to defend their protein intake when confined to low-protein diets – the protein leverage effect. As predicted, energy intake increased on the low-protein, and decreased on the high-protein diet even when macronutrient composition was disguised. To standardise the effect of fat over the leverage effect, this fat intake was kept constant at 30%, as it is well known that fat increases palatability and pleasantness (Drewnowski 2007). Carbohydrates were used as the diluting factor for

protein in the diet. The observed negative correlation between dietary protein density and energy intake therefore supports the Protein Leverage Hypothesis (Simpson and Raubenheimer 1997, Simpson, Batley et al. 2003, Simpson and Raubenheimer 2005, Sørensen, Mayntz et al. 2008, Gosby, Conigrave et al. 2011). Taken together with previous tests of PLH (Simpson, Batley et al. 2003, Simpson and Raubenheimer 2005, Gosby, Conigrave et al. 2011, Martens, Lemmens et al. 2013, Martens, Tan et al. 2014), this experimental study supports the hypothesis that an important cause of the recent global rise in obesity is a reduction in the protein density of the human diet (Simpson and Raubenheimer 2005, Simpson and Raubenheimer 2014).

The question arises of why the proportion of protein in the human diet has dropped; if humans regulate their intake to a protein target, why do not humans simply select foods with higher protein content? It should be noted that PLH does not make predictions about which foods are eaten, but only about how much energy will be eaten for a given food composition. Brooks et al. (2010) demonstrated that protein is the most expensive of the macronutrients suggesting that economic considerations might have played a role in the recent decrease in the protein density of the human diet. Such considerations could have significant implications for weight control strategies because substantial increases in energy intake is an important driver for the increase in weight observed over the recent decades (Swinburn, Sacks et al. 2009).

4.2. The Cebu study

Chapter three describes the test of PLH –for the first time- outside an experimental setting using population data: Cebu Longitudinal Health and Nutrition Survey follows a cohort of two generations. This study provided a sensitive test of this because the population is from a growing Asian city that is undergoing the nutrition transition with the associated shift in diet and nutrient intake. The analyses were longitudinal for mothers. The longitudinal findings indicate that energy from protein intake remained more constant than that from carbohydrates

or fat in a human population over a two decade period, even in different income and urbanization categories. .

Testing of PLH in a free-living human population undergoing shifts in diet and lifestyle showed interesting results in the longitudinal data analysis. Although calories from dietary protein increased slightly over time, the increase was at a slower rate than that for fat, while carbohydrates decreased slightly. These findings are consistent with the interpretation that the total amount of protein consumed is more tightly regulated than the intake of carbohydrates or fat. The dietary shift is consistent with patterns observed in other low or middle income countries (Popkin 2006, Popkin 2009, Sheehy and Sharma 2010). In the Cebu data analysis (chapter 3), calories from protein remained more constant than those from carbohydrates or fat in a human population undergoing the nutrition transition even when family income and urbanicity changed.

The socioeconomic structure of the study population changed over time with some female participants moving into higher income and urbanicity categories over the course of the study. Despite the fact that calories of dietary fat and protein increased slightly over time, total energy intake decreased among women who remained in the same income and urbanicity category. Although the population was undergoing the nutrition transition, the decreased caloric intake could be a result of the women aging over the study period. As urbanicity or income scores increased over time, the change in macronutrient intake became more steeply negative: the macronutrient intake of women in the lowest income or urbanicity category showed the smallest decrease, while women in the higher income or urbanicity categories showed the highest decrease.

Other studies (Drewnowski 2007, Darmon and Drewnowski 2008) have demonstrated that higher quality diets are, in general, consumed by better educated and more affluent people; in contrast, the results of this thesis suggest that better educated and more affluent people seem to consume a less healthy diet because they consume more fast foods. A key point of the result

is that the Philippines is a developing country, and others studies have used data from developed countries where the income is higher.

Higher education levels might also be associated with better nutrition knowledge, but this knowledge alone may not be sufficient to initiate behavioural application of healthy diets. Probably, Filipino men and women who have had a better education and have assets also had jobs that often imply limited time for food shopping and cooking. This situation could be occurring in other middle- and low-income countries. On the other hand, although socioeconomic status affects diet quality, SES seems to have non effect either total energy intake or the macronutrient composition of the diet (Darmon and Drewnowski 2008). Total energy intake correlated positively with intake of all macronutrients for males and females; also, energy from protein intake correlated positively with that from carbohydrate and fat intake. This is because in addition to a macronutrient-specific effect on calorie intake, there were also other factors driving variance in the levels of food intake, for example serving size (Hill, Wyatt et al. 2003).

Together, the work reported in this thesis extends the understanding of how dietary macronutrient content influences energy intake, by extending how PLH can be generalized to an extreme population with a history of severe childhood under-nutrition, and showing for the first time that the predictions of PLH are met in population studies outside of the experimental setting. This study supported PLH, as others studies did (Simpson and Raubenheimer 1997, Simpson, Batley et al. 2003, Simpson and Raubenheimer 2005, Weigle, Breen et al. 2005, Gosby, Conigrave et al. 2011, Martens, Lemmens et al. 2013, Gosby, Conigrave et al. 2014, Martens, Tan et al. 2014). Protein appears to exert the strongest influence on the regulation of food intake, and consequently a small shift in the proportion of dietary protein may result in a large change in the amounts of carbohydrates and fat eaten.

4.3. The nutrition transition and obesity

Protein may have sufficient leverage over human intake behaviour to explain obesity, as outlined by PLH. Nevertheless, a research priority is to see whether differences in PLH can help to explain differential susceptibility of human populations to obesity and the extent to which any differences are fixed products of differential evolution or due to developmental history. One way that PLH can play a role in promoting obesity is if populations vary in the target level of protein. In general, the higher the target level for protein the greater the effect of leverage for a given protein dietary content should be. This is because a person with a higher protein target will need to over-eat fat and carbohydrate to a greater extent to reach that target than if the protein target was lower (Simpson and Raubenheimer 2005). The results show that populations as disparate as UK, Sydney, Dutch, and Jamaican survivors of severe undernutrition all show the effect of PLH, although the environments and developmental histories differ.

Interestingly, in the USA, protein intakes are high, but the USA is particularly afflicted by obesity with one of the highest prevalences of adulthood obesity in the world (the 2012 OECD report)(Clonan, Wilson et al. 2015). Why do Americans apparently over-eat protein if protein exerts a strong effect on satiety? One possible explanation for this is that the protein leverage might be diminished in those populations in which the variety and availability of foods is greater, particularly fatty and sweet foods (Hill, Wyatt et al. 2003, Popkin 2004, Jebb 2007, Gosby, Conigrave et al. 2011). Alternatively, Americans might regulate protein intake as demonstrated for other populations (Simpson and Raubenheimer 2005, Weigle, Breen et al. 2005, Gosby, Conigrave et al. 2011) (and the Chapter 3 of this thesis), but to a higher protein target. As discussed above, and by Simpson et al. (2005), this would result in enhanced protein leverage and help to explain the high rates of obesity in USA. Analyses of the National Health and Nutrition Examination Survey (NHANES) showed that between the early 1970s and 2002-2006 in the USA increased energy intake correlated with increased absolute protein intakes, but

also a decrease in the protein:non-protein energy ratio (Austin, Ogden et al. 2011). This is consistent with an increased protein target exacerbating the protein leverage effect, thus accounting for the high rates of obesity in the USA.

The roles of protein, fat and carbohydrates

Protein intake has not previously been linked to the obesity epidemic for the following two reasons: first, protein provides only a minor part of total energy intake for humans; second, protein intake remains more constant than carbohydrates or fat intake over time and across populations and therefore does not correlate with the rise of obesity (Simpson, Batley et al. 2003, Westerterp-Plantenga 2004, Austin, Ogden et al. 2011). For this reason, numerous studies have focused on carbohydrate and fat intakes as drivers of the total energy intake, but if PLH is correct then protein intake is centrally implicated in the regulation of total energy consumption, and could be therefore an important contributor to the obesity epidemic. The thesis results support that dietary protein may have a leverage effect and significant impact on appetite and energy intake.

In the longitudinal study population, the intake of all macronutrients changed over time, and the smallest change was for protein intake, as would be expected from PLH. However, the biggest change was shown by fat, which increased, while total carbohydrates decreased; many developing countries show a decrease in total carbohydrate, particularly complex carbohydrates, while the intake of sugar has increased (Prentice 2006, Drewnowski 2007, Austin, Ogden et al. 2011). Studies have shown that in the short term increases in the proportion of fat in the diet are associated with increased energy intake, a phenomenon described as high-fat hyperphagia (Stubbs 1995). From the results of the thesis, it seems that the biggest issue in the nutrition transition -as experienced by the Cebu study population- is fat intake. If the fat content of the diet were to decrease, then so too would total energy intake. However, the relative contributions of dietary fats and sugars to the rising rates of obesity is an on-going source of

debate (Drewnowski 2007). Indeed, the results of experimental studies (Simpson, Batley et al. 2003, Gosby, Conigrave et al. 2011, Martens, Lemmens et al. 2013) (and Chapter 2) to date have shown that the protein density of the diet influence intake via its effect on carbohydrate intake. Future studies should systematically explore the interactions between protein, fat and carbohydrate.

Taste and food intake

Despite rising prevalence of obesity globally, how humans regulate the quantity and types of food items that they consume remains only partially understood. Some studies indicate that food choices are associated with taste, cost and convenience (Mela 2006, Brooks, Simpson et al. 2010). Fat-intake increase was the biggest change in the longitudinal study. Fat content has been increased in foods because fat improves palatability and its price is cheaper. Fat content has increased both in sweet and savory foods (Prentice and Jebb 2003, Drewnowski 2007), and this has not been significantly associated with food prices (Brooks, Simpson et al. 2010). Modern obesogenic environments offer easy availability of a wide variety of good-tasting, inexpensive, energy-dense foods, and the serving of these foods in large portions could promote overconsumption of energy (Hill, Wyatt et al. 2003). In this scenario, food taste has a key role in influencing total energy intake; however, data suggest that taste responses are related with macronutrient content (Drewnowski 1997). Food taste has been largely modified by the food industry; however, the human brain responds to the environment as it did hundreds years ago: sweet foods signify energy, and fatty foods signify energy storage (Cordain, Eaton et al. 2002). Sensory responses to the taste of foods help determine food preferences and eating habits; however, sensory responses alone do not predict food consumption (Drewnowski 1997). The PLH must therefore be considered in conjunction with factors that determine human choice of foods: such factors as palatability and cost influence the choice of low protein foods, whereas protein leverage explains why humans over-eat energy on these foods.

Developing countries

Surprisingly, the obesity epidemic has slowed down in some developed countries during the past three years; rates grew less than previously projected according to data from the latest OECD report (Mazzocchi and Traill 2011). However, rates remain high in developing countries; importantly, this thesis was a test of PLH in populations from two developing countries: Jamaica and The Philippines, which are undergoing a nutrition transition and where urbanization and economy are growing rapidly. Urbanization produces shifts in lifestyle (Colchero and Bishai 2008, Colchero, Caballero et al. 2008, Popkin 2009) because there is a wider choice of food sources and shifts in diet from traditional to modern forms in urban areas.

Biology clearly contributes to individual differences in weight and height, but the rapid weight gain that has occurred over the past three decades is a result of rapid environmental change interacting with human biology. However, populations from some developing countries may be particularly metabolically thrifty, with efficient storage but limited thermogenic capacities increasing the risk of obesity through overconsumption (Gluckman and Hanson 2006, Keast, Nicklas et al. 2010). Jamaican and Filipino populations showed the same protein leverage effect as UK, Dutch, and Sydney subjects; however, studies show that the degree and speed of the nutritional transition to fat-rich diets are greater for low and middle-income societies now than they were for high-income societies that began the transitions decades ago (Popkin 2002, Popkin, Bing et al. 2002). These low- middle-income societies are also experiencing greater rates of increase in the prevalence of overweight, obesity and type 2 diabetes than those experienced by high-income societies (Popkin 2002).

SES and diet quality

The relationship between socioeconomic status and obesity is complex. For example, this relationship changes as economies become more developed, with poorer people more likely

to be affected in rich countries (Su, Esqueda et al. 2012). Diet quality seems to follow a socioeconomic gradient: whereas higher quality diets are associated with high-income, energy-dense diets that are nutrient-poor are preferentially consumed by persons in lower SES groups (Darmon and Drewnowski 2008). However, the analysis of the Cebu data showed that people with high income are more likely to be affected in that developing country.

In the longitudinal study, as urbanicity or income scores increased, the change for each macronutrient – fat, carbohydrate and protein – within an income or urbanicity band became more steeply negative over time; for example, the intake of each macronutrient decreased more over time within higher urbanicity scores and higher incomes for all macronutrients. However, the degree of this effect was different for different macronutrients. Over the total population, fat and protein increased slightly; this is because people moved into higher income and urbanicity categories over time and higher income/urbanicity meant higher intake. The structure of the Cebu population has changed over time with characteristics associated with higher energy consumption. Therefore, the population intake increased despite the fact that the intake decreased for those within the same income group.

The observed trend is compounded by the fact that many giant transnational franchises present aggressive marketing campaigns to penetrate consumer bases, precisely because of increased disposable income (Popkin 2003, Popkin and Nielsen 2003, Popkin and Ng 2007). For example, the opening of supermarkets makes cheap foods readily available, however, supermarket foods are often processed, less healthy, high-fat, high-sugar and high-salt. Supermarkets also bring the advantage of convenience, a particularly attractive feature to the urban consumer in developing countries. Furthermore, studies have shown that high-protein foods are more expensive than low-protein foods; and consequently in developing countries there is a strong incentive to buy low protein foods (Brooks, Simpson et al. 2010). Therefore, the leverage effect of protein could have a huge impact on the consumption of carbohydrates and fat, and therefore on total energy intake.

“Protein leverage” and “Energy gap”

There is an urgent need to reverse the obesity epidemic. Having a specific behavioral target for the prevention of weight gain may be a key for this. Hill et al. (2003) estimated a theoretical “energy gap”, suggesting that a reduction in energy intake by 100kcal/day would prevent weight gain. The authors proposed, furthermore, that it would be possible for many people to eat 100kcal/day less without changing the types of foods they eat, by eating 15% less. Based on the present thesis, the gap could be achieved by increasing the proportion of protein. In the Jamaica study (Chapter 2), the difference in daily energy intake observed between the 10% and 15% (and between the 15% and 25%) protein diets was almost 300kcal per day. Similarly, Gosby et al. (2011) suggests that if participants maintained the increase of energy intake observed on the 10% protein diet, it would be expected that they gain one kilogram of body weight per month.

4.4. Limitations and priorities for future research

There is inevitably a trade-off between detail and generality in research. Detailed experimental studies are usually limited to a restricted population, both in numbers and characteristics (genetic heritage, age, sex, health status, developmental history etc.) of subjects, as well as the experimental setting. On the other hand, the strength of such studies is that their relative simplicity provides a good opportunity for isolating specific causes, and especially isolating causes by experimental manipulation. Extensive, population-level studies, on the other hand, are more realistic in that they are set in an ecologically more relevant context, but the large number of potentially contributing causes and interacting causes can complicate the interpretation, and are in most cases can indicate only correlation and not directly causation.

In this thesis I have attempted to combine a detailed manipulation study (Chapter 2) with an extensive population-level study to provide a more general test of the role of dietary protein content on macronutrient and energy intake that spans both detail and generality. Both

studies have their strengths and weaknesses. The logistics of the experimental study limited the number of subjects to a level that is low for testing something as complex as human intake regulation. In addition to limited number of subjects, the need for experimental control meant that the subjects were confined to an unnatural environment (residential experimental setting) for the duration of the study, rather than their usual environment. This meant that the period over which intake was measured was limited, both for measuring selected intake (the intake target) and constrained intake (testing for protein leverage). Of particular importance is the fact that the manipulations involved a limited and defined range of foods varying in macronutrient composition, meaning that complexities such as monotony could interfere with the results. One impact of this on the strength of the study is that it potentially resulted in important, unintended differences between the first stage of the study (self-selection) and the second phase (confined diets), because in the first phase the experience, including the foods, was more novel than the second phase. Future experiments should, firstly, strive to improve on these limitations, and secondly explicitly test for their effects on the outcome. Importantly, more experimental studies are needed to develop a broader idea of the circumstances (populations, foods, experimental settings etc.) under which protein leverage is a significant factor influencing energy intake in humans, and if not then how dietary macronutrient profiles influence nutrient and energy intake.

The population-level analysis (Chapter 3), by contrast, enabled me only to detect correlation, and use statistics to control, to some extent, for the large number of potentially interacting variables. Considered together, however, despite the limitations of both types of study, the fact that they both provided evidence consistent with protein leverage, and considered together with similar results from previous studies discussed throughout this thesis, suggests that this is an idea that deserves considerable more attention both in experimental and population-level studies.

An important question that is relevant to both experimental and population-level studies is how other food components, for example fibre and salt, and sub-categories of nutrients, for

example different kinds of carbohydrates (starch vs. sugar), interact with the effects of protein to influence energy intake. Protein leverage, for example, would predict that populations on low-protein diets, such as the Mediterranean and Okinawan diets, would over-eat energy, and yet these are among the healthiest dietary patterns. This be explained by the high levels of fibre in these diets, substituting for low protein to influence satiety (Raubenheimer et al. 2015).

Of particular importance are studies that bridge the gap between the simple experimental studies and uncontrolled population-level studies. For example, in the study reported in Chapter 2 subjects were experimentally constrained to eat diets of 10%, 15% or 25% protein by energy. In the real world, however, in addition to how people respond to such variation in dietary macronutrient content (e.g. increased energy intake with decreasing %P), it urgently needs to be known why people eat diets with a low protein content if this causes over- energy consumption – for example, the roles of palatability, economics, culture, sociocultural environment (e.g. exposure to fast-food outlets and sugary drinks) etc. Only by understanding this will protein leverage be an idea that can translate into real-world outcomes, because it is not through changing human biology (appetite regulation) that the problem of obesity can be solved, but through managing the modern environment in which biology is expressed to generate health and nutrition-related disease. I consider this to be the highest priority for future research, both in the detailed experimental and the broad ecological contexts.

4.5. Conclusion

The results of the studies presented in this thesis were consistent with the Protein Leverage Hypothesis playing a role in two populations undergoing the nutrition transition: low-protein diets were associated with high-energy intake in the experimental study, and protein intake remained more constant than carbohydrates or fat intake in the longitudinal study. The data presented add to previous demonstrations of protein leverage in UK (Simpson, Batley et al. 2003), Australia (Gosby, Conigrave et al. 2011), and the Netherlands (Martens, Lemmens et

al. 2013, Martens, Tan et al. 2014) and suggest that protein leverage is widespread among humans. In conclusion, based on data from two population studies of diet and food intake, protein density of the human diet may very well be part of the obesity epidemic (Simpson and Raubenheimer 2005, Simpson and Raubenheimer 2014), but more research is needed to establish the generality and details of its relevance in realistic settings.

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