CAPSAICINOIDS – A POTENTIAL ROLE FOR WEIGHT MANAGEMENT

S J Whiting

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Stephen John Whiting

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Abstract

The prevalence of overweight and obese individuals has risen dramatically in populations around the world over the last 30 years (Popkin et al., 2012) representing a rapidly growing burden to public health services (Wang et al., 2011). Reliance on lifestyle modification, although initially promising, has proven to be unsuccessful over the longer term (Barte et al., 2010) and there are currently a lack of successful treatment options.

Capsaicinoids are a bio-active group of compounds, naturally occurring in the fruit of the plant from the genus *capsicum*. Initial research suggests these compounds may have beneficial effects on weight loss outcomes when ingested (Lejeune et al., 2003). A systematic review of the available literature on capsaicinoids found evidence that ingestion may increase energy expenditure by around 210kJ/day and lipid oxidation by around 20%. Ingestion may also reduce energy intake although evidence has been conflicting and the size of the effect unclear.

To further aid understanding, a meta-analysis was undertaken involving intervention trials assessing the effects of capsaicinoids on energy intake. Analysis suggested capsaicinoid ingestion prior to a meal reduced *ad libitum* energy intake energy intake by 251kJ (60kcal) per meal (95% confidence interval of 337 – 166kJ) $p < 0.001$. Caution should be applied to this result however, due to the small size of the reduction and the short term nature of the trials involved. Longer term trials are needed to assess potential changes in body composition as a result of capsaicinoid interventions.

To this end, a six-week placebo control intervention study was conducted to assess changes in body fat in 60 Caucasian women. Results of a sensitivity analysis found a small, statistically significant decrease in body fat percentage (0.64%, $p = 0.022$) and total body fat (0.67kg, $p = 0.007$) in the intervention group. However, the robustness of these findings are called into question by the results of an interaction analysis which of observed no significant difference between placebo control and intervention groups over time for these outcomes. The effect was also small and therefore longer term supplementation would be required to produce a medically beneficial changes in body composition.

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Abbreviation

ANOVA	-	Analysis of Variance
BMI	-	Body Mass Index
BAI	-	Body Adiposity Index
BAT	-	Brown Adipose Tissue
EE	-	Energy Expenditure
GLP-1	-	Glucagon-Like Peptide-1
NHLBI	-	National Heart Lung and Blood Institute
NICE	-	National Institute for Health and Care Excellence
SD	-	Standard Deviation
SNS	-	Sympathetic Nervous System
TRPV1	-	Transient Receptor Potential Cation Channel Subfamily V Member 1
WAT	-	White Adipose Tissue
WHO	-	World Health Organisation

Chapter 1 – Introduction

1.1 Background

Overweight and obese populations represent a rapidly growing financial and health threat to many societies (Dietz, 2015). Often described as an epidemic, obesity rates began rising in the USA in the 1980s and continues today around the world. In 2008, 1.5 billion adults (aged 20 and older) were overweight; of these around 500 million were obese (WHO, 2011).

Obesity has been found to decrease health-related quality of life (Fontaine and Barofsky, 2001) and overall life expectancy (Peeters et al., 2003). In addition to this, obese patients are at an increased risk of coronary heart disease (Colditz et al., 1995, Rimm et al., 1995), type 2 diabetes (Colditz et al., 1995), strokes (Rexrode et al., 1997), hyperlipidaemia (Rabkin et al., 1997), hypertension (Witteman et al., 1989) and several cancers (Calle et al., 2003, Calle and Kaaks, 2004).

The exact costs of obesity to the NHS in the UK are complex to assess, but research estimates that the costs were £3.2 billion in 2007 (Allender and Rayner, 2007) and have the potential to rise to £5 billion by 2030 (Wang et al., 2011). In the current economic climate, with strain on government spending (Swords, 2014), the potential costs of treating the co-morbidities of a preventable disorder are prohibitive, with research suggesting that the healthcare costs for obese patients are approximately 30% higher than normal-weight individuals (Pelone et al., 2012).

Increasing obesity rates reflect a sustained period of positive energy balance for the affected population. While it is widely accepted that increasing energy expenditure and reducing energy intake form the basis for obesity management, reversing this trend has proven very difficult (Gortmaker et al., 2011). Biological, behavioural and environmental factors all interact to affect energy balance and body weight regulation (Hill, 2006) and reliance on diet and exercise interventions alone have proved relatively unsuccessful over the long term, with weight regain a major problem (Wu et al., 2009).

A number of biological changes occur in the body following weight loss making maintenance more difficult and increasing the likelihood of weight regain (MacLean et al., 2011). One of the main problems is a reduction in resting metabolic rate following weight reduction. As an individual loses weight, their basic energy requirements also reduce, as their metabolism slows down. This means further reductions in energy intake/increases in energy expenditure are required and it means maintenance of a lower body weight is difficult to sustain (Curioni and Lourenco, 2005). In addition to this, after weight loss, changes in the circulating levels of several hormones (including leptin and ghrelin) involved in the homeostatic regulation of body weight occur (Strohacker et al., 2013). These changes encourage weight regain after diet induced weight loss and have been observed to persist over a long-term period (one year) (Sumithran et al., 2011). There may also be physiological changes in the hypothalamus in response to calorie deficiency in some individuals (Sainsbury and Zhang, 2010). The hypothalamus is an area of the brain largely responsible for signalling hunger and influencing energy intake levels. Changes in this region of the brain following weight reduction may make maintaining this loss more problematic (Berthoud and Morrison, 2008).

To counteract this, the pharmaceutical industry has attempted to develop drugs to assist weight loss, with limited success. Most of the drugs that have entered the market for treating obesity were originally developed to treat psychiatric diseases (Adan et al., 2008). Targets have included reducing appetite, reducing food absorption (especially lipids) and increasing energy expenditure. While drugs have been successful in influencing these targets (such as sibutramine, fenfluramine and dexfenfluramine), side effects have often proved too severe. A number of drugs have had to be withdrawn from the market, particularly because of cardiovascular complications, and the options for treatment are currently limited (McGavigan and Murphy, 2012).

Therefore, the development of safe weight loss aids is of particular interest to doctors, health services and individuals looking to lose weight. Researchers have begun looking at a number of naturally occurring compounds that may be able to produce similar anti-obesity effects, but with less severe side effects. Trials show there may be potential to aid people with weight loss for amongst others, caffeine (Boozer et al., 2002), catechins from green tea (Yang et al., 2012) and capsaicinoids from chilli

peppers (Lejeune et al., 2003). However, patent legislation provides little monetary incentive for the pharmaceutical industry to develop naturally occurring compounds for medical applications.

This research aims to review and assess current research into the potential weight loss effects of capsaicinoids, the group of compounds found in chilli peppers that causes their pungent flavour when eaten, and to further investigate these effects by carrying out a placebo controlled intervention trial.

1.2 Aims

The aims of the research projects are as follows:

1) To perform a systematic literature search of human intervention trials investigating the effects of capsaicinoids on weight management outcomes, collate and analyse findings publish the results as a systematic review paper.
2) Undertake a meta-analysis summarising the results of intervention trials measuring the effects of capsaicinoids on energy intake and publish results.
3) Plan and perform a double-blind, placebo controlled intervention study to investigate the effects of capsaicinoids on body fat in human subjects.

1.3 Structure of the Thesis

The thesis begins with a literature review (chapter 2) starting with current obesity trends, prevalence and a critical appraisal of available treatments. Also featured will be an assessment into current research involving capsaicinoids, including structure, uses and potential regarding weight loss outcomes, along with current understanding of their potential mechanisms of action. Chapter 3 of the thesis presents a meta-analysis of intervention studies conducted to further understanding regarding capsaicinoids' potential effect on energy intake following ingestion. Chapter 4 details an intervention trial undertaken to assess capsaicinoids' potential effect on fat loss in Caucasian women, with the methodology and results presented. Chapter 5 initially presents a discussion into the intervention trial, including critical analysis of potential issues and then a discussion of the thesis as a whole and what could be done to further enhance study in this area.

Chapter 2 – Literature Review

2.1 Introduction

This chapter will give an overview of current obesity problem, in terms of prevalence, past and future trends; the health problems associated with it and the estimated financial cost. The review will then explore current treatment practices and finally propose evidence for an alternative weight management aid, namely capsaicinoids, a naturally occurring group of chemicals found in chillies.

2.2 Obesity and Weight Management

The increasing prevalence of overweight and obesity, measured by body mass index (BMI), is a worldwide health concern. In a systematic analysis of epidemiological studies from 199 countries, 1·46 billion adults worldwide were estimated to be overweight in 2008, and of these 502 million were obese (Finucane et al., 2011). The effects of consistently high prevalence of obesity on population health are far-reaching; societies face being burdened by premature mortality, morbidity associated with many chronic disorders, and reduced health-related quality of life (Wang et al., 2011). With some researchers arguing that increasing BMI is a worldwide pandemic (Roth et al., 2004), that could reverse life-expectancy gains in high-income nations (Olshansky et al., 2005).

2.2.1 Health burden of Obesity

The rising prevalence of obesity is a worldwide health concern because excess weight gain within populations forecasts an increased burden from several diseases. Obesity is a major risk factor for cardiovascular disease (CVD) including coronary heart disease (CHD), coronary artery disease, stroke, congestive heart failure and atrial fibrillation (Guzzardi and Iozzo, 2013). Obesity and overweight are highly prevalent in patients with CHD. The prevalence of overweight and obesity in the general U.S. population are 66% and 36% respectively (Ogden, 2012), whereas the prevalence overweight and obesity rates in patients with CHD entering cardiac rehabilitation are more than 80% and 44%, respectively (Audelin et al., 2008). From 1996 to 2006 there

was a 33% increase in the prevalence of obesity in individuals with CHD (Audelin et al., 2008).

A significant association between obesity and increased risk for ischemic stroke has been documented in both genders and in different ethnic populations (Caucasians, African American, Chinese and Japanese) (Yatsuya et al., 2010, Lee et al., 2011, Bazzano et al., 2010). Research also suggests the severity of strokes are increased with obesity (Osmond et al., 2009). Based on these findings, the American and Stroke Associations recommend the treatment of obesity for both primary (Goldstein et al., 2011) and secondary stroke prevention (Furie et al., 2011).

While obesity is an independent risk factor for the development of CVD (Wilson et al., 2002) it also predicts an elevated risk through its association with a cluster of risk factors termed the metabolic or insulin resistance syndrome (Grundy et al., 2005). These associated risk factors include hypertension, hypertriglyceridemia, low levels of high density lipoprotein cholesterol, abdominal obesity, insulin resistance and type 2 diabetes mellitus (Garg et al., 2014).

Studies have demonstrated a clear association between obesity and type 2 diabetes (Guzzardi and Iozzo, 2013). In a cross-sectional survey of adults with diabetes, the lowest prevalence for diabetes was found in individuals with normal weight (BMI <25). The prevalence of diabetes increased throughout the range of obesity classes. Nearly a quarter of adults with diabetes have poor glycaemic control and nearly half of the individuals with diabetes are considered obese (Nguyen et al., 2011b). The prevalence of obesity among diabetic individuals is much higher than the reported prevalence of obesity in the general adult population. The prevalence of obesity among adults with diabetes was found to be 49.1% of a sample of the US population (Nguyen et al., 2011b), while the prevalence of obesity among the general US population has been reported to be 32.2% in adult men and 35.5% in adult women (Flegal et al., 2012).

Obesity also has a strong influence on cancer mortality, with epidemiological data from the past 25 years pointing to obesity as a cause of approximately 14% of cancer deaths in men and up to 20% of cancer deaths in women (Calle et al., 2003). A major review of weight and cancer incidence using obesity prevalence data from Europe concluded, that obesity was a cause of 11% of colon cancer cases, 9% of

postmenopausal breast cancer cases, 39% of endometrial cancer cases, 25% of kidney cancer cases, and 37% of oesophageal cancer cases (Vainio et al., 2002). In addition, data from the American Cancer Society suggested that overweight and obesity were related to increased mortality from liver cancer, pancreatic cancer, non-Hodgkin's lymphoma, and myeloma (Calle and Kaaks, 2004). The effect of obesity on cancer reflects both an increase in incidence and an increase in mortality among those diagnosed with cancer (Wolin et al., 2010).

Excess body weight also contributes to non-fatal but disabling disorders such as osteoarthritis (Guh et al., 2009). Moreover, rapidly expanding evidence suggests that excess body weight is linked to many additional disorders, including benign prostate hypertrophy (Nichols et al., 2011), infertility (Withrow and Alter, 2011), asthma (Wang et al., 2008), and sleep apnoea (Sassi, 2010). In addition to this, maternal obesity has been linked to an increased risk of congenital anomalies, further contributing to the health burden (Stothard et al., 2009). Because in many populations the prevalence of obesity is greater at a younger age than in previous generations, present trends in obesity project a growth in the percentage of the population living with chronic disease (Wang et al., 2011). Some researchers have hypothesised that the continued increase in life expectancy achieved by medical and public health advances during the past century may be reversed by increasing obesity prevalence (Muennig et al., 2006).

2.2.2 Financial Burden of Obesity

The multiple chronic and acute health conditions related with overweight and obesity afflicted societies not only by negatively affecting the health-related quality of life of their populations but also by incurring considerable financial costs to the individuals affected and to society as a whole (Anandacoomarasamy et al., 2009). These costs are sustained predominantly from increased the health-care costs and lost economic productivity of the individuals involved (Yang and Hall, 2008). The medical costs of obesity represent the monetary value of health-care resources required to manage obesity-related disorders, including the costs incurred by excess use of ambulatory care, hospitalisation, drugs, radiological or laboratory tests, and long term care (Wang et al., 2011).

In a systematic review of the direct health-care costs of obesity, it was estimated that obesity accounted for up to 2.8% of healthcare expenditure; although the authors noted that the studies involved were generally very conservative, such that the actual amount was likely to be higher (Withrow and Alter, 2011). Analysis from the USA found that compared normal-weight individuals, obese patients incur 46% increase in inpatient costs, 27% more clinical and outpatient costs, and an 80% increase in spending on prescription drugs (Finkelstein et al., 2009).

In addition to medical costs, economies incur substantial indirect costs from obesity as a result of decreased years of disability-free life, increased mortality before retirement, early retirement, disability pensions, work absenteeism and reduced productivity (Wolfenstetter, 2012). Several studies suggest that the monetary value of lost productivity is several times larger than medical costs (Trogdon et al., 2008, Finkelstein et al., 2010). Researchers in the USA reported that annual missed workdays ranged from 0·5 more days in men who were overweight to 5·9 more days in men who were classified as grade III obese (BMI ≥40 kg/m²) than in men of healthy weight (Finkelstein et al., 2010).

Evaluating the costs from the health consequences of obesity is complex; costs are affected by a number of factors such as changes in the economy, food system and population demographics. Estimation of the cost from lost productivity is especially challenging because of the scarcity of data and the assumptions needed for the labour market structure (Lehnert et al., 2013). Some researchers argue that inclusion of unrelated future costs distorts decision making about resource allocation (Garber and Phelps, 1997, Lee, 2008); others advocate the inclusion of unrelated medical costs in life-years gained in all economic evaluations of preventive interventions, although they acknowledge the practical challenge and scarcity of comprehensive data for doing so (Rappange et al., 2008). Regardless, the NHS cannot continue to fund the rising cost of treating obesity and its co-morbidities.

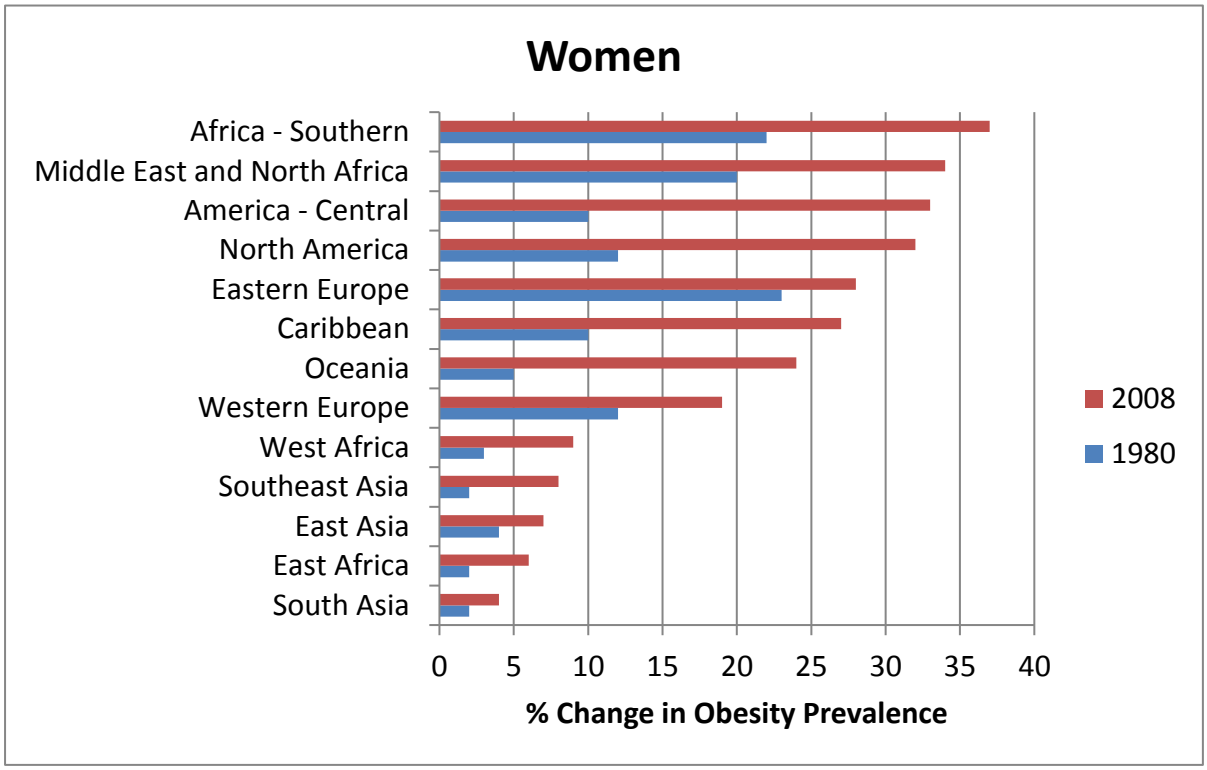
2.2.3 Prevalence and Trends

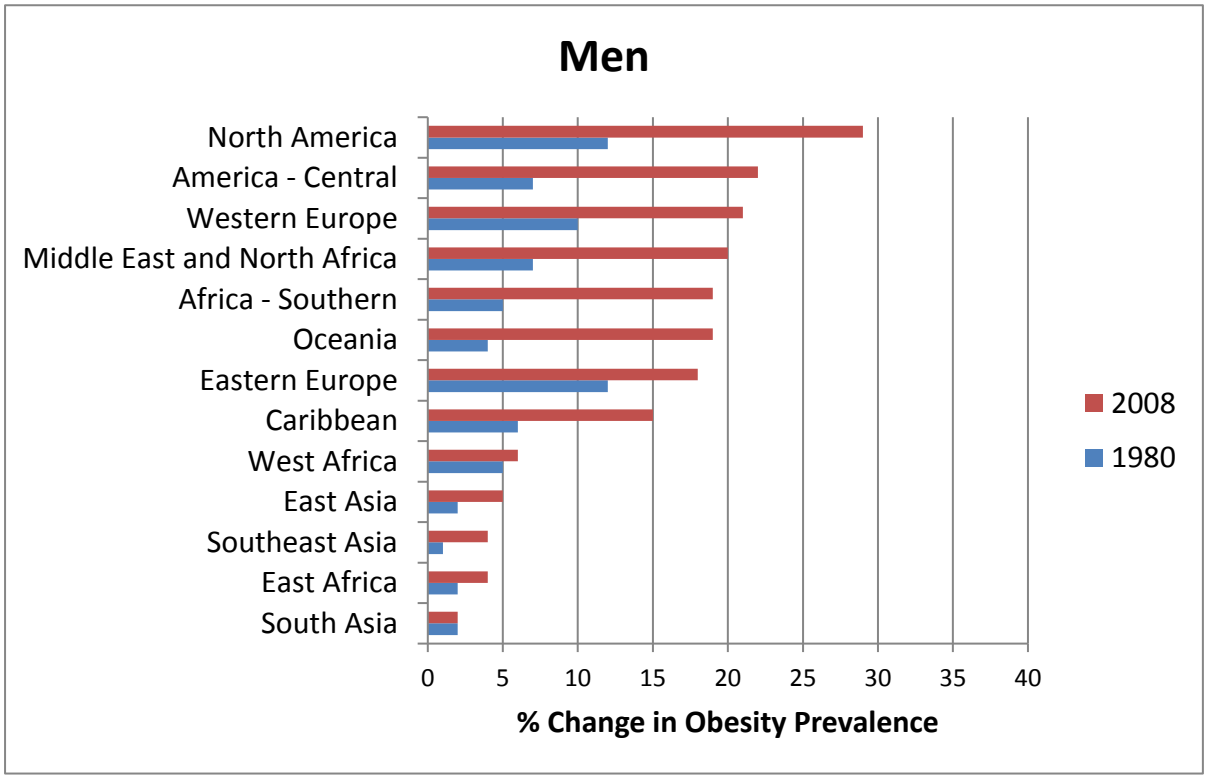
Initially observed in high income countries of North America and Western Europe, obesity has now become a major contributor to the global burden of disease (Olshansky et al., 2005). The worldwide increase in the incidence of obesity and

related chronic diseases has been caused by dramatic changes in lifestyles and eating habits that promote a positive energy balance and lead to weight gain (Kearney, 2010). This has been largely driven by economic development, global trade liberalisation and rapid urbanisation of world economies (Hawkes, 2006). Positive energy balance occurs when an individual's caloric intake exceeds their energy expenditure, leading to weight gain. Although economic development has led to many benefits such as improvements in food security and life quality, it has also lead to an increase in the consumption energy dense foods and reductions in physical activity, which has triggered the observed increases in overweight and obesity (Fuster and Kelly, 2010).

The global effect of the obesity epidemic was formally recognised in by the World Health Organisation during a special consultation in 1997 (James, 2008). In the past 15 years a large body of evidence has accumulated documenting the increases in prevalence of obesity across the world (Malik et al., 2013). These figures suggest an estimated 500 million adults were obese (BMI > 30 kg/m^2), which represents 10-14% of the world's population (see figure 2.1). Globally between 1980 and 2008, obesity prevalence rose from 4.8% to 9.8% in men and from 7.9% to 13.8% for women (Finucane et al., 2011).

Figure 2.1 Charts showing in prevalence of obesity among women and men in 1980 and 2008 from select regions around the world. Adapted from (Finucane et al., 2011).



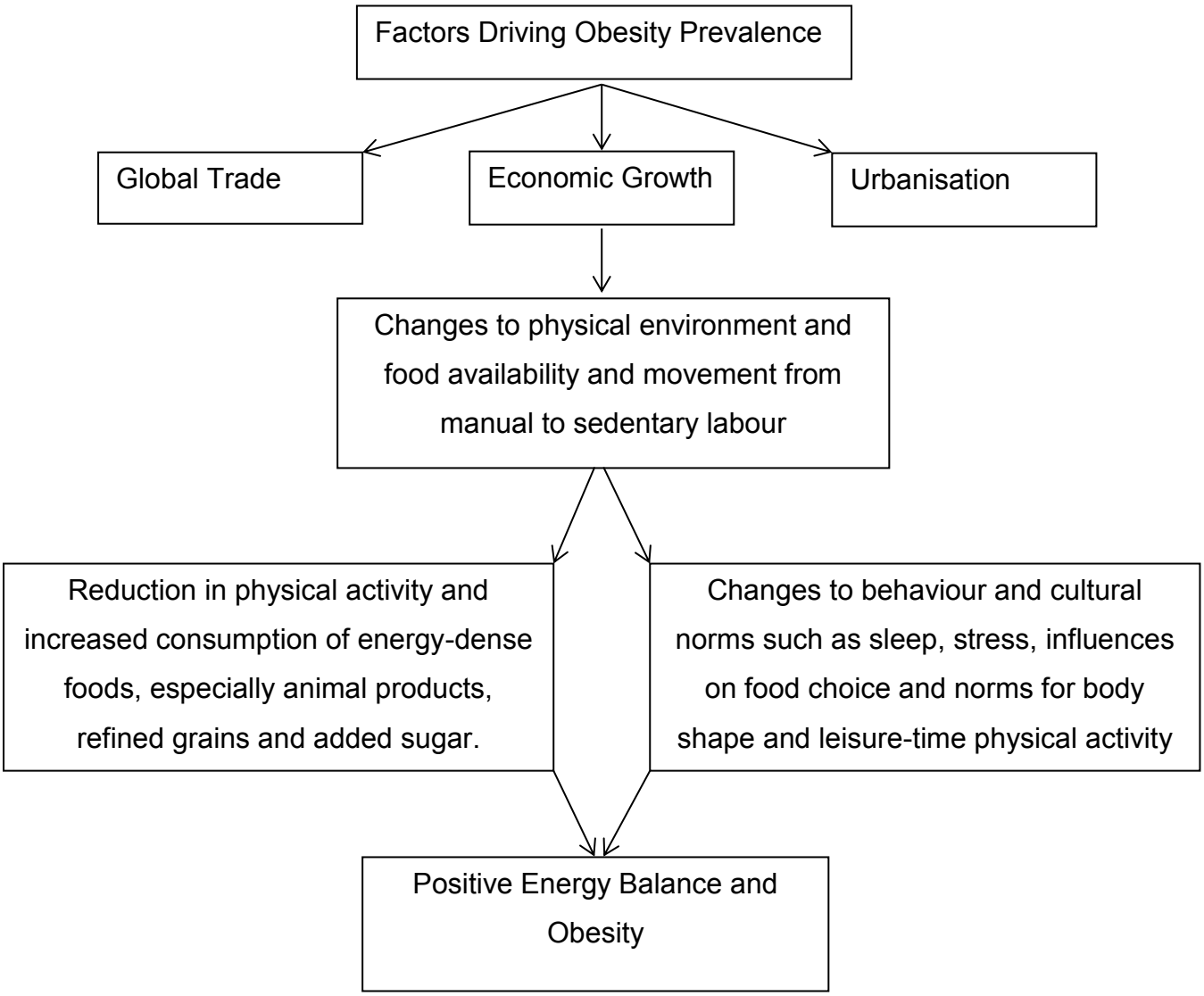


Although figures vary widely across different countries and geographical regions (see figure 1.7), in the USA for example in 2009-10, 35.5% of men and 35.8% of women were classified as obese (Flegal et al., 2012), in China in 2010 the prevalence of obesity (BMI > 28) 11.9% for men and 12.1% for women (Li et al., 2012). As seen from figure 1.7, obesity rates are high in developed regions such as North America and Europe, but are also high in emerging economic regions such as the Middle East and Southern Africa. Low obesity rates are seen in Asia and less the developed economies of West and East Africa (Seidell, 2014).

2.2.3.2 Effect of Income and Socio-economic Status on Obesity Prevalence

The prevalence of obesity correlates positively with the initial stages of economic growth and development in low income countries (Ezzati et al., 2005). Populations often undergo rapid nutritional and lifestyle transitions, while having limited access to health services and education (Popkin et al., 2012). Research analysing global patterns of nutritional risks in relation to economic development in 100 countries, showed that BMI increased rapidly in relation to national income (Ezzati et al., 2005). This is of particular concern as over the next three to four decades, global *per capita* income is projected to rise at a rate of >2% per year; and in low income countries, this increase is expected to be even more rapid (Kearney, 2010).

Figure 2.2 Flow chart representation of the relationship between economic growth and obesity (Malik et al., 2013)



Driving this increase are the factors of global trade liberalization, economic growth and urbanization; which promote the development of obesity through changes to the physical environment and food choice. Movement away from physical labour to sedentary activities, creating so called 'obesogenic' environments, which affect physical activity levels, diet, behaviours and cultural norms (see figure 2.2) (Malik et al., 2013). All of these effects combine to create a state of positive energy balance that promotes obesity (Popkin et al., 2012).

2.2.3.4 Levelling Off in Prevalence Rates

There is evidence that growth obesity prevalence may be levelling off in some parts of the world. A systematic review, including the results from the 52 studies (Rokholm et al., 2010), found a clear tendency towards a stabilisation of the obesity levels in children and adolescents from Australia, Europe, Russia and the USA and a decrease was identified in Japanese children and adolescents. In contrast, strong increases were observed in Chinese and Vietnamese children and adolescents. The tendency in European adults is, however, more ambiguous, as increases were still reported in conscript data from Austria, Denmark and Sweden. (Rokholm et al., 2010). What is clear, even with a levelling off of an increase in obesity prevalence, level remain high, posing a threat to both global health and global economies (Wang et al., 2011).

2.2.3.5 Future Trends

Over the next two decades, the largest proportional increase in the number of adults who are overweight or have obesity is expected to occur in low-income and middle-income countries, where estimates range from increases of 62 – 71% for overweight and 205 - 263% for obesity (Kelly et al., 2008). In many of these countries, the increase in obesity prevalence has occurred rapidly, as a result of economic development (Popkin and Gordon-Larsen, 2004). In many of these emerging economies, people who are overweight exist along those who and suffer malnutrition, not only in the same communities but even in the same households; this is termed the ‘dual burden household’ (Doak et al., 2005). A number of countries therefore, have to manage the dual burdens of obesity and related chronic diseases, while still dealing with the problems of underweight and under-nutrition (Malik et al., 2013). This creates the potential for obesity to create major strain on health services around the world.

2.2.4 Body Mass Index

Virtually all measures of obesity prevalence are based on the calculations of individual’s body mass index. BMI has a number of advantages: the medical profession have been educated in its use and it has made significant inroads into the general public consciousness (Prentice and Jebb, 2001). It is a relatively simple and repeatable measure, with a minimum of equipment needed, (a balance and a

stadiometer), meaning it can easily be performed in numerous locations around the world (Deurenberg et al., 1991) and the errors in measurement tend to be small (Mei et al., 2002).

However, because the BMI depends upon weight and the square of height, it ignores basic scaling laws whereby mass increases to the 3rd power of linear dimensions. Hence, larger individuals, even if they had exactly the same body shape and relative composition, always have a larger BMI (Taylor and Marchand, 2010). BMI generally overestimates adiposity on those with more lean body mass (e.g., athletes) and underestimates excess adiposity on those with less lean body mass (Deurenberg et al., 2001). Also BMI may not provide an accurate assessment of increased mortality risk. A 2005 study showed that overweight people had a similar relative risk of mortality as normal weight people as defined by BMI, while underweight and obese people had a higher death rate (Flegal et al., 2005). Therefore, the widespread use of BMI is a limitation of current obesity research, which may not provide accurate health assessment of population groups.

2.2.5 Current Approaches to Obesity Treatment

Relatively modest weight loss of 5% to 10% of initial weight has been shown to produce significant health benefits (Moyer, 2012). Researchers have found reduced cardiovascular disease risk factors such as lower systolic and diastolic blood pressure, blood triglycerides and an improved HDL/LDL cholesterol profile (Look AHEAD Research Group, 2010); prevention or delay of the development of type 2 diabetes (Knowler et al., 2002); and improvements in other obesity related health consequences including sleep apnoea (Foster et al., 2009), urinary incontinence (Phelan et al., 2012), mobility (Rejeski et al., 2012) and symptoms of depression (Rubin et al., 2010). Research from a large (5,145 participants), multi-centre (19 clinics), long-term trial (13.5 years) found that larger weight losses were associated with greater improvements in risk factors. The magnitude of weight loss at was highly related to the improvements in blood pressure, glycaemic control, and lipids. There also was no evidence that a patient's weight at baseline affected the amount of improvement that occurred with a given percentage change in body weight (except for HDL cholesterol) (Wing et al., 2011).

To reverse the effects of obesity, people are usually treated by the medical profession in a three tier progression. Firstly, people are counselled on evidence-based lifestyle approaches that include diet, physical activity and behaviour change therapies. Secondly the use of pharmacological agents such Orlistat (sold under the trade names Xenical and Alli) have been used as adjuncts to lifestyle modification. Finally, it may be appropriate for severally obese individuals who have not been responsive to initial treatments to undergo bariatric surgery (Kushner, 2013)

2.2.5.1 Lifestyle Treatments

Comprehensive lifestyle modification programs typically provide weekly individual or group treatment sessions designed to modify eating and activity habits (Wadden et al., 2007). Lifestyle modification, also referred to as behavioural weight control, includes 3 primary components: diet, exercise, and behaviour therapy (NHLBI, 2011). Interventions tend to be long term with the aim of maintaining behaviour change, in larger trials these interventions have lasted for 24 weeks in the Diabetes Prevention Program (3200 participants) (Knowler et al., 2002) and six months in the Look AHEAD study (5100 participants) (Look AHEAD Research Group, 2003).

2.2.5.1.1 Diet

Participants initially follow a calorie restricted diet to induce a medically significant level of weight loss is achieved, a different diet may then be followed to maintain losses (Wadden et al., 2012). Much research has been carried out into the ideal combination of macronutrients to achieve weight loss, however studies show weight loss is primarily dependent on reducing total calorie intake, not the proportions of carbohydrate, fat, and protein in the diet (Sacks et al., 2009). Incorporating meal replacements into the diet is a common strategy (Keogh and Clifton, 2005); meal replacements are foods that are designed to take the place of a meal or snack while at the same time providing nutrients and good taste within a fixed caloric limit (Wadden et al., 2009). For the maintenance diet to achieve long-term weight loss, most obese individuals must consciously restrict their energy intake, whether by reducing portion sizes, decreasing the energy density of the diet, counting calories (or specific macronutrients), or some combination of these approaches (Wadden et al., 2012).

2.2.5.1.2 Physical Activity and Exercise

In addition to reducing caloric intake, participants in weight loss interventions are also encouraged to burn more calories. Research has highlighted the distinction between physical activity and planned exercise. Whereas physical activity consists of any bodily movement that increases energy expenditure, e.g. activities of daily living like walking, climbing stairs, gardening, etc., exercise is defined as planned, structured, and repetitive bodily movement done to improve or maintain one or more components of physical fitness (Armstrong, 2006). Studies have demonstrated that lifestyle activities are as effective as structured exercise programs in improving overall fitness and maintaining weight loss (Swift et al., 2013). However, physical activity alone is of limited benefit in inducing weight loss; most individuals cannot find the time or motivation to engage in the high volume of activity required for significant weight loss (for example 35 miles of walking a week is required to lose around 0.5 kg/week) (Donnelly et al., 2009). Several research investigations have however, shown physical activity to be critical for long-term weight management (Catenacci and Wyatt, 2007, Tate et al., 2007).

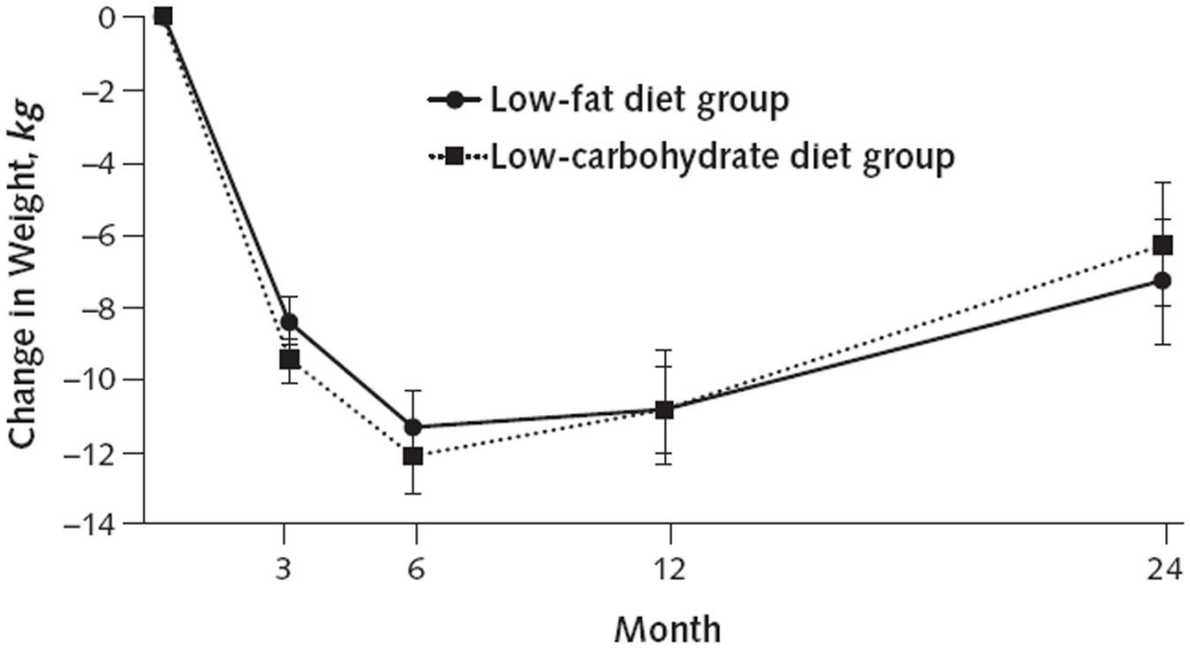
2.2.5.1.3 Behaviour Therapy

Behaviour therapy as part of lifestyle modification programs, refers to a set of principles and techniques for helping obese individuals modify eating, activity, and thinking habits that contribute to their excess weight (Brownell, 2004). This recognizes that body weight is affected by factors other than behaviour, including genetic, metabolic, and hormonal influences, which likely predispose some persons to obesity and set the range of possible weights that a given individual can achieve (Wadden et al., 2007). Key components of behaviour therapy include setting specific goals for behaviour change that specify what an individual will do, and when, where, how, and for how long he or she will engage in the behaviour (Wadden and Foster, 2000). Perhaps the most useful strategy in achieving lifestyle goals is to include self-monitoring (Burke et al., 2011). Patients are asked to track their food intake, physical activity, and weight throughout treatment. The benefits of tracking include having real-time data on dietary intake as it relates to caloric and other nutritional goals, allows reflection and planning of diet, introduces restraint, and provides information to share

with the provider; the major drawback however is that many participants find this a burden, especially over the longer term (Cadmus-Bertram et al., 2013)

With intensive lifestyle treatments, a majority of obese participants in clinical trials lose 7% to 10% of their initial weight after one year (Carvajal et al., 2013). However, results from research trials are generally far better than those attained by patients in primary care settings. Studies using low-intensity counselling have failed to demonstrate clinically meaningful mean weight loss (Wadden et al., 2013). Regardless of initial weight loss success, the major problem is often that longer-term weight maintenance is difficult, with around 80% of people losing weight failing to maintain this loss over multiple years (Wing and Phelan, 2005). For example, figure 2.3 shows this weight loss-weight gain cycle of 307 participants over a 2 year period following an intervention featuring 20 weekly lifestyle modification sessions, followed by 10 every other week interventions (Foster et al., 2010).

Figure 2.3 Graph showing weight loss and gradual regain over a two year period following a 40 week intervention; adapted from (Foster et al., 2010).



With continued lifestyle treatment, weight regain can be minimised but is difficult to eliminate entirely, and this features a significant ongoing cost (Ades and Savage, 2010). The need for constant vigilance to sustain behaviour changes in the face of

biologic and environmental pressures to regain weight emphasizes the challenges faced by even the most motivated patients who have achieved weight loss. Thus, there is a need for adjunctive therapies that can help patients who are not able to lose or sustain sufficient weight loss to improve health with lifestyle interventions alone (Kushner, 2013).

2.2.5.2 Pharmacotherapy

Medications for obesity have traditionally fallen into two major categories: appetite suppressants (anorexiants), and gastrointestinal fat blockers. Appetite suppressing medications have targeted three monoamine receptor systems in the hypothalamus: noradrenergic, dopaminergic and serotonergic (Kushner, 2008). In the 1930s amphetamines were first used as anorexiants, but due to their addictive nature were replaced with several related compounds, five of which had approval for use in the USA by the 1960s (Kushner, 2013). Despite their success in assisting weight loss, they have developed a legacy of dubious safety, with a number of drugs removed from the marketplace due to safety concerns (Williams, 2010). For example fenfluramine was withdrawn from the U.S. market in 1997 after reports of heart valve disease (Weissman, 2001) and dexfenfluramine was withdrawn from markets around the world following multiple concerns about cardiovascular side effects (Gardin et al., 2000).

Such withdrawals have left options for pharmacological treatment of obesity limited, though the following drugs are approved for use. Orlistat, sold under the brand name ‘Xenical’ and ‘Alli’, was first approved for use as an anti-obesity drug in 1999 (Ahmad and Mahmud, 2010). It is a gastrointestinal lipase inhibitor which, when taken 3 times per day during or up to 1 hour after meals, leads to the excretion of approximately 30% of ingested fat (Kumar et al., 2013). It is available in prescription (120mg) and over-the-counter (60mg) strengths. The mean 12-month weight reduction attributable to orlistat 120 mg taken 3 times per day is modest: among adults participating in behavioural weight control programs and prescribed a lower-fat diet (30% of calories from fat) and a multivitamin, participants taking Orlistat lost on average 3.4 kg (3.1% of initial weight) more than participants taking placebo (Sjöström et al., 1998). Because Orlistat leads to increases in undigested stool triglycerides, it can cause considerable gastrointestinal adverse effects, which may result in patients to discontinue therapy

(Filippatos et al., 2008). Indeed, despite being approved for indefinite treatment of obesity, among those prescribed Orlistat 120 mg clinically, fewer than 10% take it for at least 1 year and less than 2% of patients are prescribed the medication for 2 years (Hampp et al., 2013).

Lorcaserin is a serotonergic agent like fenfluramine and dexfenflurame, but with improved functional selectivity. It is thought to decrease food intake through the pro-opiomelanocortin system of neurons (Smith et al., 2010) and has been approved for use as a prescription anti-obesity drug in 2012 in the USA (Miller, 2013). In two large trials (both over 3000 patients) participants received low-intensity nutritional and exercise counselling along with 10mg of lorcaserin. The active intervention decreased body weight modestly, by approximately 3.2 kg (3.2% of initial body weight) more than placebo after one year (Smith et al., 2010, Fidler et al., 2011).

Phentermine and topiramate is combination drug that contains a catecholamine releaser (phentermine) and an anticonvulsant (topiramate). Weight loss was observed as an unintended side effect of topiramate during clinical trials for epilepsy (Ben-Menachem et al., 2003). The combination drug was developed to reduce adverse events incorporating lower doses in a fixed combination formulation which has been tested in two large trials (Yanovski and Yanovski, 2014). In the EQUIP trial, with 1267 participants, those given the top dose had a mean 1-year weight loss of 10.9% vs 1.6% of initial weight compared to the placebo group (Allison et al., 2012). In the CONQUER trial with 2487 participants, one-year mean weight loss was 8.1 kg (7.8%) with the recommended dose and 10.2 kg (9.8%) with the top dose vs 1.4 kg (1.2%) with placebo (Gadde et al., 2011). It should be noted however, that withdrawal rates were high at 40% in the EQUIP trial 31% in the CONQUER trial.

Although lorcaserin and the phentermine/topiramates combination therapy have been approved for use in the USA, both drugs have failed to be approved in Europe. There are concerns that despite their success as anti-obesity drugs lorcaserin may increase the potential risk of psychiatric disorders (such as depression) and valvulopathy; with the phentermine and topiramates combination drug there are concerns about it increasing cardiovascular risk (Woloshin and Schwartz, 2014). Given the history of approval and withdrawal of anti-obesity drugs due to long-term safety, researchers

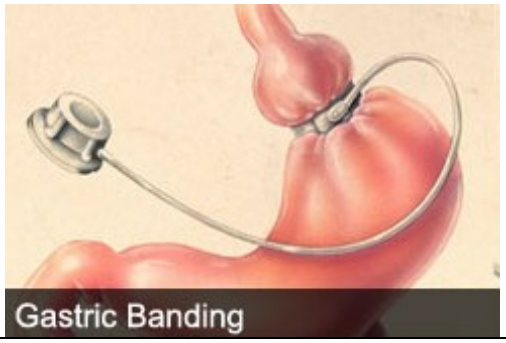
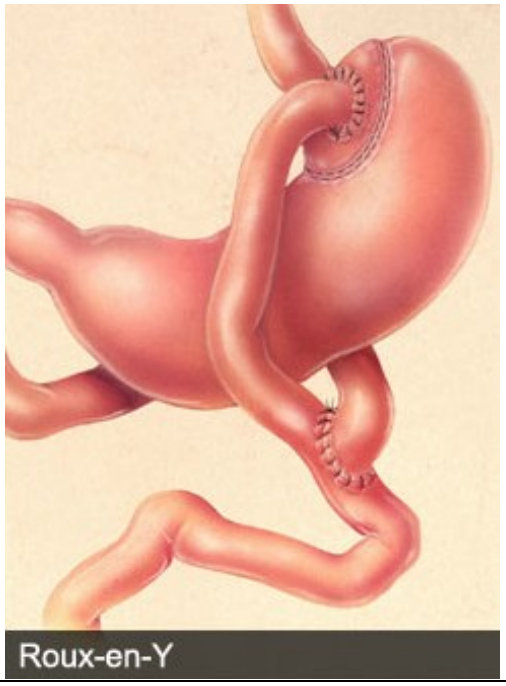
have argued it is unclear if these drugs will remain on the market in the USA in the future (Wolfe, 2013). The lesson from the withdrawal of previous anti-obesity drugs is that uncommon but serious adverse effects may become apparent only when a drug is used in larger populations or for longer periods of time than in the preapproval trials (Lauer, 2012). Thus, pharmacological treatment options for obesity remain very limited, particularly in Europe where only Orlistat is approved and people rarely stick to its use long term due to gastro-intestinal side effects.

2.2.5.3 Bariatric Surgery

The use of bariatric surgery to treat morbid obesity has increased dramatically over the past two decades. An estimated 350,000 bariatric procedures were performed globally (63% in US/Canada) in 2008 compared to fewer than 5,000 procedures between 1987–1989 (DeMaria et al., 2010). Typically patients have to have failed to keep weight off with lifestyle interventions before they become eligible for bariatric surgery (NICE, 2006).

Currently, 90% of procedures are performed laparoscopically; the two most common procedures being adjustable gastric banding (42% of procedures), Roux-en-Y gastric bypass (40% of procedures) (Buchwald and Oien, 2013) (see table 2.1 for further details). Adjustable gastric banding is a simpler, shorter procedure that does not carry a long-term risk of malabsorption. It also leads to a lower number of serious complications but consistently produces smaller reductions in weight and a higher requirement for overall reoperations than Roux-en-Y gastric bypass (Padwal et al., 2011).

Table 2.1 A table describing the most common types of bariatric surgery, adapted from (Padwal et al., 2011).

Procedure	Description	Illustration
Adjustable gastric banding	A band encircling the proximal stomach and connected to a subcutaneous port/reservoir is inserted. The band remains initially deflated and is then progressively inflated via the subcutaneous port during subsequent postoperative outpatient visits to achieve gastric restriction and weight loss.	
Roux-en-Y gastric bypass	The proximal stomach is separated from the distal stomach to form a small, restrictive gastric pouch. The gastric pouch is connected to mid-jejunum (thus forming the alimentary or roux limb) which has been diverted away from the biliopancreatic secretions. The length of small intestine distal to site at which the roux limb and the biliary limb join is termed the 'common limb' and represents the intestinal area where biliopancreatic secretions mix with ingested food and most absorption occurs. The alimentary limb is typically 100 cm in length and the biliopancreatic limb 30 to 50 cm in length.	

The clinical benefits of bariatric surgery in achieving weight loss and improving metabolic comorbidities have largely been attributed to changes in the physiological responses of gut hormones and adipose tissue metabolism (Ionut et al., 2013). Metabolic effects resulting from bypassing the foregut include altered responses of ghrelin, glucagon-like peptide-1 and peptide YY3–36 (le Roux et al., 2007). Additional effects on food intake and body weight control may be attributed to changes in vagal nerve signalling; the loss of fat mass, particularly visceral fat, is associated with multiple metabolic, adipokine, and inflammatory changes that include improved insulin sensitivity, glucose disposal and reduced free fatty acid flux (Laferrère et al., 2011).

Mean percent weight loss at 5 years is estimated to be 20% to 25% of initial weight for gastric banding (Dixon et al., 2012) and 25% to 30% of initial body weight at 5 years for Roux-en-Y gastric bypasses (Vest et al., 2013).

However, there are risks associated with bariatric surgery and complications are frequent. A study of insurance claims of 2522 patients who had undergone bariatric surgery showed 22% suffered complications during the initial hospital stay and that total rose to 40% in the subsequent six months. Approximately 30% of bariatric surgery patients will develop a nutrition-related complication, typically a macronutrient or micronutrient deficiency or both, at some point following their operation (Dalcanale et al., 2010). Protein-energy malnutrition is one of the most serious nutrition complications of bariatric surgery. This complication may be a consequence of reduced intake of protein such as red meat, which is poorly tolerated after bariatric surgery, or the development of other gastrointestinal problems that result in poor oral intake and excessive weight loss (Fujioka et al., 2011). Such side effects have led to surgery only being used in the most obese patient (average BMI of those undergoing surgery exceeds 45 kg/m^2) and considered inappropriate for those of lower BMIs by many health professionals and researchers (Consortium, 2009).

2.3 Potential Role of Bio-active Compounds in Obesity Treatment

As described previously, the primary method for managing body weight has been a three tier approach. Starting with intensive lifestyle interventions, followed by the use of medications to either increase energy expenditure or reduce energy intake and finally the use of bariatric surgery. Using intensive lifestyle interventions, consisting of dietary restriction and physical activity accompanied with behaviour therapy have proven to be largely ineffective. Even with long term interventions (around six months), once the intervention period finishes compliance with improved lifestyle is poor. The result is generally a transient phase of weight loss followed by a return on the trajectory towards obesity.

Anti-obesity drug development programmes have been littered with false starts, failures in clinical development, and withdrawals due to adverse effects that were not fully appreciated at the time of launch (Rodgers et al., 2012). As a result, current

options for treatment are particularly limited, particularly in Europe where only Orlistat is available to prescribe and few people stick with taking it over the long term. Once established, obesity, like hypertension or dyslipidaemia, requires long term treatment. Therefore, medications for obesity treatment must be viewed through the lens of long-term use when evaluating their safety and efficacy (Yanovski and Yanovski, 2014).

Most forms of bariatric surgery have a relatively rapid and substantial beneficial effect on weight loss and health markers such as hyperglycaemia (Buchwald et al., 2009). It has been argued by some researchers and surgeons therefore, that reservations towards surgery should be set aside and that it should be used as a 'first line' treatment option that can cure obesity (Zimmet and Alberti, 2012). However, the risk of side effects with bariatric surgery are substantially higher than all other medical treatments, with some researchers suggesting that if it were a medical treatment rather than a surgical one, it would by now have been halted by regulatory authorities, and may only be appropriate for the most obese patients (Pinkney, 2011).

Although the increasing prevalence of obesity is associated with an obesogenic environment, that encourages over-eating and discourages physical activity, it is now recognized that there exist a genetic susceptibilities which also play an important role in determining the extent to which an individual resists or is prone to obesity (Blundell et al., 2005, Blundell and Cooling, 2000). In addition, it has been established that in response to reduced calorific intake and weight loss, there is an accompanying decrease in energy expenditure, which is in part due to loss of lean body mass, and in part due to an enhanced metabolic efficiency (Major et al., 2007). Such reductions in thermogenesis may also persist well beyond the phase of weight loss (Rosenbaum et al., 2008) and have been demonstrated in both the resting and non-resting elements of energy expenditure (Dulloo et al., 2004).

These mechanisms may make a significant contribution to weight regain and obesity relapse (Rosenbaum et al., 2008). Considering the lack of success of obesity treatments, new approaches that could diminish these compensatory mechanisms and offset genetic predispositions to obesity and weight regain after weight loss are therefore desirable (Hursel and Westerterp-Plantenga, 2010). Due to the lack of success of pharmacological agents in providing a clear physiological benefit at an

acceptable level of side effects, attention has turned to naturally occurring bio-active ingredients. The potential to produce positive effects on thermogenesis, fat oxidation and appetite have led to an active search for compounds that could provide such as effect without the long-term problems (particularly on cardiovascular risk factors) associated with previously developed pharmacological agents (Yuliana et al., 2014). One such compound may be capsaicinoids, a compound naturally occurring in chillies (Whiting et al., 2012).

2.4 Chillies

2.4.1 Origin

Chillies are the fruits of plants from the genus *Capsicum*, which are members of the night shade family, Solanaceae (Perry et al., 2007). The plants originate from South America, with research proposing they evolved in dry, mountainous regions in either Bolivia or Peru (Walsh and Hoot, 2001). There are between 23 and 27 wild species, 5 of which have been domesticated (see table 2.2), and from these 5 species over 2000 cultivars have been bred (Tewksbury et al., 2006). Chillies available for human consumption have a huge range of characteristics with variations in pungency, colour, shape, flavour and size. Despite their trait differences however, most chilli cultivars commercially grown in the world belong to the species, *C. annuum* (Bosland, 1992).

Table 2.2 Domesticated chilli species and common varieties (McGee, 2004)

Species	**Commonly Consumed Cultivars**
Capsicum annum	Bell pepper, jalapeno, cayenne
Capsicum chinense	Naga, habanero, scotch bonnet
Capsicum frutescens	Tasbasco, peri, Thai peppers
Capsicum pubescens	Rocoto
Capsicum baccatum	Aji

Chilli has been part of the human diet since at least 7500 BC and archaeological evidence indicates they were domesticated more than 6000 years ago in south-western Ecuador (Perry et al., 2007). They have since become an important food source and it is estimated that one quarter of the world's population consume chillies

every day (Cordell and Araujo, 1993). They are also the most widely grown spice worldwide, with production and consumption levels 20 times that of black pepper, the other major pungent spice (McGee, 2004).They do not however feature prominently in the traditional Northern European and North American diet, where consumption rates tend to be much lower (Ludy and Mattes, 2011a).

The main unique feature of the fruit is its pungency or 'spicy' sensation when eaten; this derives from a group of chemicals unique to the plant – capsaicinoids. They occur throughout the fruit, but are concentrated in the placental tissue, which holds up to 90% of capsaicinoids; lower levels are found in the pericarp and lower still in the seeds (which are held in the placental tissue) (Cisneros-Pineda et al., 2007). Evidence suggests the plant evolved these molecules as a protection mechanism for seeds (Arora et al., 2011).

2.5 Capsaicinoids

Unique to chillies, capsaicinoids are the group of chemicals that provide the fruit with its most distinctive property – pungency. Although more than 10 different chemicals exist, the most prominent forms are capsaicin and dihydrocapsaicin, typically accounting for almost 90% of capsaicinoids found in chilli peppers (Meghvansi et al., 2010). Capsaicin's chemical formula and chemical structure were first determined in 1919 (Nelson, 1919), and in 1961, similar substances were isolated from chili peppers by Japanese chemists who named them capsaicinoids (Kosuge et al., 1961). The amount of capsaicinoids produced by chillies can vary widely. Genetics play a major role, with *Capsicum chinense* containing the highest values and are therefore the most pungent (the hottest variety is the naga pepper) (Meghvansi et al., 2010). Growing conditions also play an important role, such as high temperatures and water availability, as does time of harvest (Reilly et al., 2001).

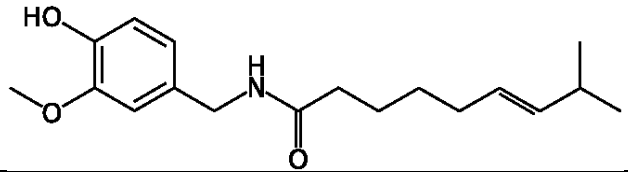
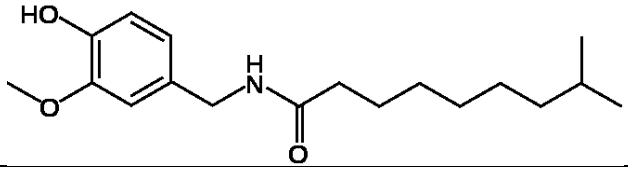
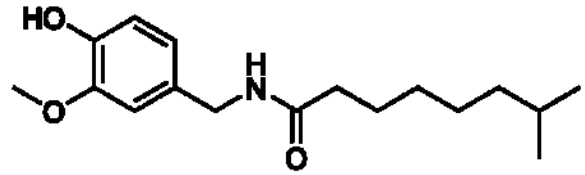
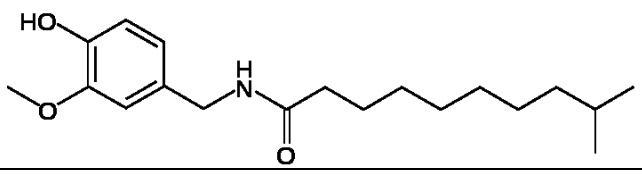
Research has observed that South American wild chillies are mainly eaten by local bird populations, which do not appear to be deterred by the presence of capsaicinoids. Local rodents however, are deterred from eating chillies containing capsaicin, both in the wild and lab conditions (Tewksbury and Nabhan, 2001). When the rodents consume non-pungent chillies, the digestive system damages the seeds, resulting in

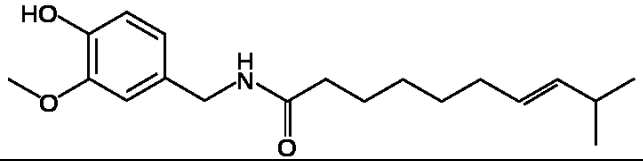
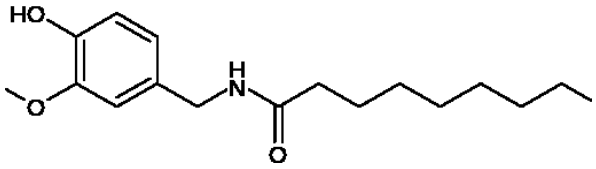
a zero germination rate. Consuming by the local bird population however, resulted in similar rates of germination to that of control seeds planted directly from the fruit (Tewksbury and Nabhan, 2001) suggesting capsaicinoids may selectively choose who consumes the fruit and therefore who aids seeds dispersal.

2.5.1 Chemical Structure and Properties

All capsaicinoids are acid amides, with carbon chains (9-11 atoms in length), branched-chain fatty acids and vanillylamine (see table 2.3). The major differences between the various capsaicinoid molecules are the length of the aliphatic side chain, the presence or absence of a double bond and the branching point (Díaz et al., 2004). Most are pungent, but there are also non-pungent capsaicinoids, such as the ω-hydroxycapsaicin (Ochi et al., 2003). Isolated pure capsaicinoids are crystalline, lipophilic, colourless and odourless alkaloids, which are fat, oil and alcohol soluble (Reyes-Escogido et al., 2011). Their pungency is very strong; with one part in 100,000 detectable by tasting (Arora et al., 2011).

Table 2.3 Structures of most common capsaicinoid compounds (Luo et al., 2011).

Name	Chemical Structure	Chemical Formula
Capsaicin		$C_{18}H_{27}NO_3$
Dihydrocapsaicin		$C_{18}H_{29}NO_3$
Nordihydrocapsaicin		$C_{17}H_{27}NO_3$
Homodihydrocapsaicin		$C_{19}H_{31}NO_3$

Homocapsaicin		$C_{19}H_{29}NO_3$
Nonivamide		$C_{17}H_{27}NO_3$

Capsaicinoids are synthesized naturally in the placenta of chilli fruits from enzymatic condensation of vanillylamine and different-sized fatty acid chains which are elongated by a fatty acid synthase. This process is regulated by the capsaicin synthase enzyme acting specifically on fatty acid chain length, which requires Mg2+, ATP, coenzyme A and vanillylamine, the phenolic portion, formed from phenylalanine as a product in the phenylpropanoid pathway, while the fatty acid is formed from the amino acids valine or leucine (Reyes-Escogido et al., 2011). In vitro synthesis of capsaicinoid molecules is also possible. Capsaicinoid production via cell or tissue culture can be augmented with the addition of biosynthetic pathway precursors and intermediaries such as phenylalanine, ferulic acid and vanillylamine (Ramachandra and Ravishankar, 2002). Given the importance of capsaicinoids to the food and pharmacological industries (see section 1.2.3), recent research has begun to focus on the genetic factors that influence capsaicinoids synthesis (Datta and Jana, 2010, Finger et al., 2010).

2.5.2 Measuring Capsaicinoid Pungency

Pungency was first assessed by organoleptic tests, the most famous of which, the Scoville heat test, was first introduced by Wilbur Scoville in 1912. The original method involved an overnight alcohol extraction from the pepper, then a tasting panel testing increasing dilutions of the extract until the pungency was undetectable (Scoville, 1912). The dilution factor was then assigned to the chilli as ‘Scoville heat units’; the more pungent an extract is the more it can be diluted, the higher the score (pure capsaicin’s score would be 16 million). The scoring system has been adapted to modern chemical analysis and so Scoville heat units are still regularly used to grade pepper pungency today (see table 2.4).

Table 2.4 Scoville Heat Units (SHU) for variety of peppers (McGee, 2004)

Pepper Variety	Species	SHU score
Naga	Capsicum chinense	300,000 - 800,000
Habanero	Capsicum chinense	200,000 - 300,000
Birds eye	Capsicum annuum	100,000 - 125,000
Tabasco	Capsicum frutescens	30,000 - 50,000
Cayenne	Capsicum annuum	30,000 - 50,000
Chile de Arbol	Capsicum annuum	15,000 - 30,000
Jalapeno	Capsicum annuum	7,000 - 25,000
Ancho	Capsicum annuum	2,500 - 3,000
Bell pepper	Capsicum annuum	Less than 200

Organoleptic tests have since been replaced by more modern chemical analysis; the most widely used method being high-performance liquid chromatography (HPLC), which offers adequate accuracy and precision. The standard analytical method uses a 4.6 mm × 250 mm C-18 column with a mobile phase consisting of a mixture of acetonitrile, dioxane, water, methanol, and perchloric acid depending on the sample concentration, with either absorption UV detection or fluorescence detection used depending on sample size (Latimer, 2012). The drawbacks to HPLC method include the expense of columns and solvents, analysis time, and production of chemical wastes that require proper disposal (Davis et al., 2007). The majority of modern published research expresses capsaicin content in mg/g rather than Scoville heat units (SHU).

2.5.3 Current Uses

Humans have used capsaicinoids for purposes other than food consumption for hundreds of years; studies have revealed that chillies *(Capsicum* solanaceae) were incorporated into a number of medicinal preparations by the indigenous Mayan inhabitants of Central America (Cichewicz and Thorpe, 1996).

In 1878, a burning sensation and hyperaemia was observed when a chilli extract was applied to human skin (Toh et al., 1955). Early research with animals revealed a fall in blood pressure, increase in salivary and gastric secretion and increased intestinal activity after intravenous injections of chilli extracts (DeLille and Ramirez, 1935). Understanding the actions of capsaicinoids led to the discovery of its receptor, transient receptor potential vanilloid subfamily member 1 (TRPV1 also known as the capsaicin receptor) which is central to many of the observed effects capsaicinoids have on the human body (see section 2.8.1 for an in depth description of this) (Cioffi, 2007). Despite research into several different areas of health, capsaicinoids are currently only used in medicine for pain relief.

2.5.3.1 Pain relief

Pain relief is the most studied medical application of capsaicinoids (Luo et al., 2011). What makes capsaicin unique is that the initial excitation of sensory neurons is followed by a lasting refractory state, referred to as 'desensitisation' (Gerner et al., 2008). This action is a result of capsaicin's effect on the transient receptor potential cation channel subfamily V member 1 (TRPV1, which occur in cells throughout the body), which helps the body detect heat or warmness (Ramsey et al., 2006); capsaicin is an agonist of TRPV1 and reduces its heat activation threshold (Knotkova et al., 2008). Capsaicin activated TRPV1 go into a long refractory state and thus a previously excited neuron is resistant to various stimuli ranging from mechanical pressure to endogenous and exogenous pain and pro-inflammatory agents (Szallasi and Blumberg, 1999).

2.5.3.2 Cancer

Evidence has emerged in the last two decades that capsaicinoids may have anti-cancer properties, and have displayed anti-tumour activity in both in vitro and in vivo studies (Sharma et al., 2013). In the cultured cells, capsaicin was able to block breast cancer cell migration and kill prostate cancer cells, and dihydrocapsaicin was reported to induce the autophagy in human colon cancer cells (Oh et al., 2008, Thoennissen et al., 2010, Yang et al., 2010). In the animal models, oral consumption of capsaicin was able to decrease the size of breast cancer tumours by 50% in mice, inhibit the development of pre-neoplastic breast lesions by up to 80% and direct injection of

capsaicin led to 80% reduction in size of the tumours (Thoennissen et al., 2010). Research from epidemiological studies however, has suggested that consumers of large amounts of chillies were at higher risk of gastric cancer than non-consumers and that metabolites of capsaicin (such as the reactive phenoxy radicals) may attack the DNA and trigger the mutagenicity and malignant transformation (Báez et al., 2010). Therefore, capsaicin has been described as a 'double edged sword' with both carcinogenic and chemo-preventive properties, and as such has rarely been considered to treat cancers in a clinical setting (Luo et al., 2011).

2.5.3.3 Cardiovascular Effects

There is evidence that capsaicinoids could have potentially beneficial effects on the cardiovascular system (Harada and Okajima, 2009, Peng and Li, 2010) which is rich in capsaicin-sensitive sensory nerves (particularly the TRPV1 receptor), and may play a role in regulating cardiovascular function through the release of multiple neurotransmitters (Zhou et al., 2010). Capsaicin has been shown to inhibit platelet aggregation (Adams et al., 2009, Raghavendra and Naidu, 2009), which is a critical factor in arterial thrombosis (Chuang et al., 2013). Capsaicinoids also have antioxidant properties; in vitro, it has been reported that capsaicin and dihydrocapsaicin were able to increase the resistance of LDL to oxidation by delaying the initiation of oxidation and/or slowing the rate of oxidation (Ahuja et al., 2006). In vivo, capsaicin treatment reduced serum total cholesterol and lipid peroxide level in rats fed on a high fat diet (Manjunatha and Srinivasan, 2007), and regular consumption of chilli for 4 weeks increased the resistance of serum lipoproteins to oxidation in adult men and women (Ahuja and Ball, 2006).

2.5.3.4 Gastro-Intestinal Effects

Like the cardiovascular system, the gastrointestinal system is also rich in capsaicin-sensitive sensory nerves (particularly TRPV1 receptor), which are believed to play an important role in the maintenance of gastrointestinal mucosa integrity against injurious interventions (Peng and Li, 2010). The gastro-protective effects of capsaicinoids were demonstrated in different animal models of gastric mucosal injury, induced by hydrochloric acid, ammonia, ethanol, aspirin or indomethacin (Mózsik et al., 2007, Szolcsanyi and Bartho, 2001). Additionally, capsaicin has been shown to alter brush

border membrane permeability, associated with increased microvilli length and perimeter, resulting in an increased absorptive surface of small intestine (Prakash and Srinivasan, 2010) and increased mineral absorption in rats (Prakash and Srinivasan, 2013).

However, detrimental effects on GI tract on prolonged exposure of high doses of capsaicin have been reported. A high dose of capsaicinoids trial led to the exhaust of neurotransmitters and the damage of capsaicin-sensitive sensory nerves, which may have harmful effects on the gastrointestinal system in rats (Wang et al., 2005). It seems, capsaicinoids exert either beneficial or detrimental effects on gastrointestinal mucosa depending on the dose and/or duration of treatment (Raybould, 2013).

Table 2.5 Recent emerging evidence of other physiological actions of capsaicinoids

Condition	Key Finding	Reference
Alzheimer's disease	Capsaicin protected against the formation of Alzheimer's lesions in rats.	(Jiang et al., 2013)
Asthma	Treatment of TRPV1 channel in mice reduced asthma symptoms.	(Rehman et al., 2013)
Dermatological conditions	21 day capsaicin treatment reduced symptoms of psoriasis vulgaris.	(Yu, 2011)
Fibromyalgia	6 weeks of topical capsaicin treatment significantly reduced symptoms.	(Casanueva et al., 2013)
Skeletal muscle hypertrophy	Capsaicin mimics mechanical load-induced intracellular signalling events resulting in muscle hypertrophy	(Ito et al., 2013)

2.5.4 Toxicity and Adverse Effects

The dosage at which capsaicinoids are applied is clearly crucial to their use. At high does they are a highly irritant substance causing burning or stinging pain to the skin. Eye exposure leads to intense tearing, pain, conjunctivitis and blepharospasm. If it is

ingested in large quantities it can cause nausea, vomiting, abdominal pain, and burning diarrhoea (Hayman and Kam, 2008). The oral toxicity of capsaicin has been investigated in mice and rats; with the lethal dose required to kill 50% of the population being 118.8 mg/kg for male and 97.4 mg/kg for female mice, and 161.2 mg/kg for male and 148.1 mg/kg for female rats. Major toxic symptoms were salivation, erythema of skin, staggering gait, bradypnea and cyanosis. Some animals showed tremor, chronic convulsion, dyspnoea and lateral or prone position and then died 4 to 26 min after dosing. Survivors recovered within 6 hours in mice and 24 hours in rats (Saito and Yamamoto, 1996). Other research with dogs suggested pure capsaicin is rapidly eliminated, induces transient tachycardia and hypertension, does not alter the duration of cardiac action potentials, and causes only very minimal organ toxicities (Chanda et al., 2005).

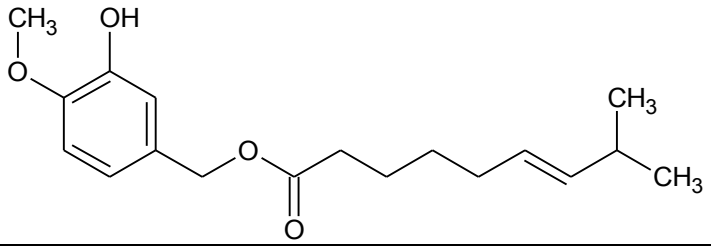
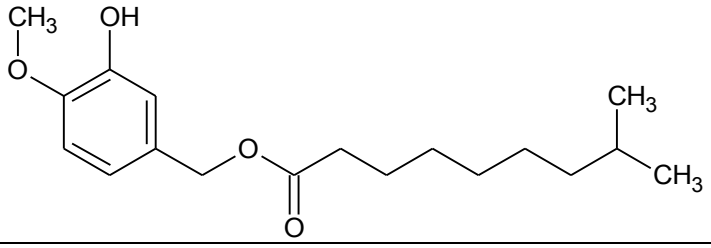
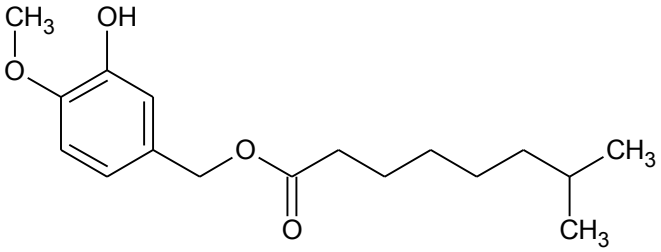
In studies with human subjects, approximately 54% of patients using capsaicin experienced one or more local adverse events (compared to 15% with placebo) (Sawynok, 2005). The initial burning side effect of capsaicin cannot be reliably prevented with the use of topical local anaesthetics and this is a major limitation in the use of capsaicin as a topical therapy (Hayman and Kam, 2008). Another adverse effect of capsaicinoids is coughing, which occurred in 8% of patients using 0.075% cream whereas none occurred with 0.025% cream. Capsaicin has been extensively used in cough testing because it produces a dose-dependent and reproducible cough response; with TRPV1 receptors present on sensory airway nerves being crucial to the mechanism of the cough reflex (Dicpinigaitis and Alva, 2005). Adverse gastro-intestinal effects following oral consumption were reported by 11% of participants in a trial with 91 participants and a high capsaicin dosage (135mg/day) (Lejeune et al., 2003), suggesting this is around the upper limit of oral ingestion of capsaicin supplements.

2.6 Capsinoids

Recently, a number of capsaicin analogues, named capsinoids, have been the focus of research. They occur naturally in high levels in a capsicum cultivar named ‘CH-19 Sweet’, which has low levels of capsaicinoids (Kobata et al., 1998). Capsinoids include capsiate, dihydrocapsiate, and nordihydrocapsiate (Kobata et al., 1999); they bear a

similar structure (an aliphatic hydroxyl group in vanillyl alcohol with a fatty acid) with capsaicinoids, however, the central linkage in both group compounds is different (He et al., 2009) (see table 2.5 for structures). They have become of interest to researchers as they possess many of the same bio-active properties of capsaicinoids, without the same level of pungency. Like capsaicinoids, much of capsinoids' physiological influence come from their agonistic effect on TRPV1 receptors (Iida et al., 2003).

Table 2.5 Structures and chemical formulas of the most common capsinoids (Luo et al., 2011)

Capsiate		$C_{18}H_{26}O_4$
Dihydrocapsiate		$C_{18}H_{28}O_4$
Nordihydrocapsiate		$C_{17}H_{26}O_4$

Studies carried out to evaluate the toxicity of capsinoids suggest they are less toxic to mammals than capsaicinoids. Research in rats has shown that administration of dihydrocapsiate by gavage at a dose of 100 or 300 mg/kg/day for 13 weeks did not see any significant changes in clinical signs, body weight, food consumption, water intake, ophthalmology, urinalysis, haematology, or blood chemistry (Bernard et al., 2008, Kodama et al., 2010).

It has been shown that capsiate, different from capsaicin, is highly lipophilic and easily broken down in the normal aqueous conditions, which may account for, at least

partially, the non-pungent property and lower toxicity of capsiate (Kawabata et al., 2006). In contrast to capsaicin, capsiate did not induce any significant responses when applied to the skin surface, eye or oral cavity of mice, suggesting that capsiate requires direct access to nerve endings to exhibit its effects (Iida et al., 2003). Other research has shown that capsinoids do not stimulate the nociceptors in the mouth of humans, meaning upon consumption there is not the same pungent flavour sensation (Hursel and Westerterp-Plantenga, 2010). This ability has led to suggestions that capsinoids may make a good alternative to capsaicinoids, particularly as some people are adverse to the heat sensation in both the mouth and the gastro-intestinal tract, especially when consumed in large quantities (Yoneshiro et al., 2012).

2.7 Capsaicinoids, Capsinoids and Weight Management

The potential weight management effects of capsaicinoids were first observed in rats when capsaicin treatment stimulated epinephrine secretion from the adrenal medulla which resulted in a rapid but transient elevation of the respiratory quotient, thus showing an increase in energy expenditure (Kawada et al., 1988). Similar effects were also observed in human subjects when a 25% increase in the metabolic rate was observed after chilli sauce and mustard sauce were added to a meal (Henry and Emery, 1986).

2.7.1 Mechanisms of Action

Three potential actions of capsaicinoids and capsinoids have been identified that may lead to weight loss, weight maintenance or other beneficial health outcomes. Namely an increased energy expenditure, increased lipid oxidation and decreased energy intake. The potential mechanisms of actions for these effects will be discussed in this section.

2.7.1.1 The Transient Receptor Potential Vanilloid Type 1 Ion Channel

It was first observed in the 1950s that capsaicin activated a subpopulation of nociceptive sensory nerves (Porszasz and Jancso, 1959). The receptor was then identified and cloned in 1997 (Caterina et al., 1997) and since then has been renamed the transient receptor potential vanilloid type 1 ion channel (TRPV1). Many the known actions of capsaicinoids' and capsinoids' are mediated by their effects on the TRPV1

(Gharat and Szallasi, 2007). Capsaicin contains a vanillyl moiety (Fujiwake et al., 1980); a chemical structure that which is believed to cause the activation of the TRPV1 channel (Mandadi and Roufogalis, 2008).

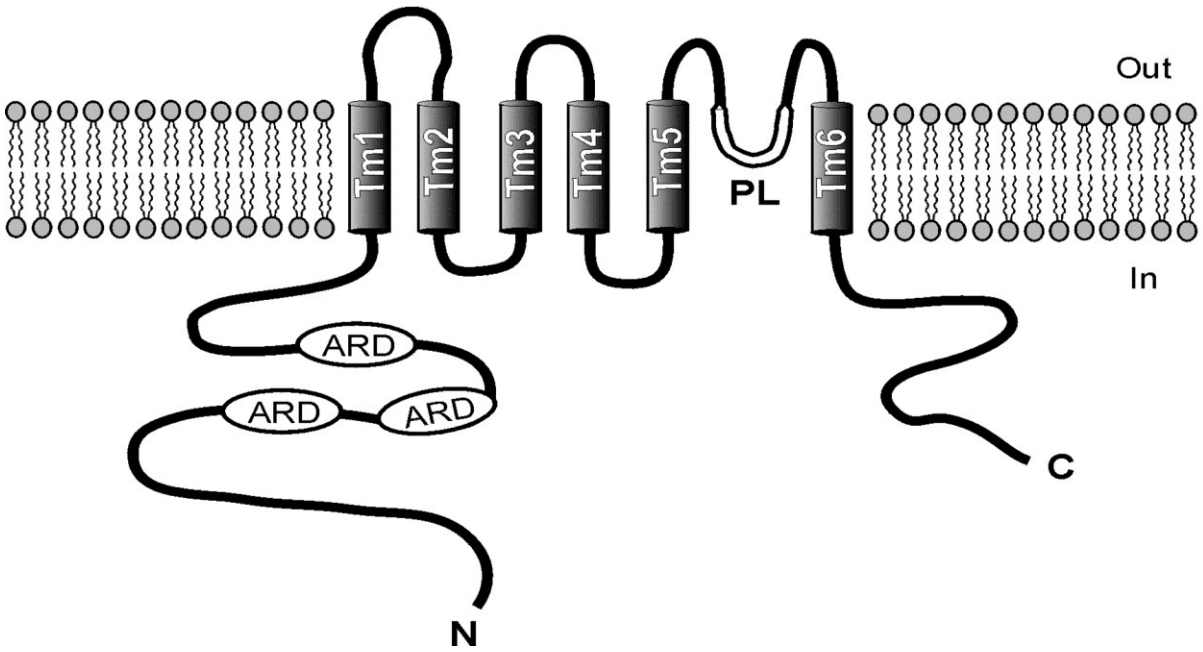
Ion channels such as the TRPV1 are located within the plasma membrane of nearly all cells and many intracellular organelles. They are often described as narrow, water-filled tunnels that allow only ions of a certain size or electrical charge to pass through; a characteristic known as selective permeability. A typical channel pore is just one or two atoms wide at its narrowest point and is selective for specific types of ions, such as sodium, potassium or in the case of the TRPV1, predominantly calcium. Ions often move through the segments of the channel pore in single file nearly as quickly as the ions move through free solution. In many ion channels, passage through the pore is governed by a "gate", which may be opened or closed in response to chemical or electrical signals, temperature, or mechanical force (Hille, 2001). Activation of TRPV1 results in increased openness of the ion channel and subsequently increased ion movement in and around the channel (Liu et al., 2006).

TRP channels respond to changes in various physical stimuli such as heat or pressure (Nilius and Appendino, 2011). TRPV1 was the first TRP channel shown to respond specifically to increased temperatures (Caterina et al., 1997). Interestingly, TRPV1 seems to be continually active at a low level and hence it is thought it acts as a molecular thermometer. It has been proposed therefore, that the main function of TRPV1 is to maintain body temperature (Gavva et al., 2007). This putative function explains why some TRPV1 agonists such as capsaicinoids can cause a change in body temperature, and also why TRPV1 antagonists induce considerable hyperthermia in several species (Holzer, 2008). An increase from normal room temperature to about 45 °C has been shown to lead to a pronounced increase in the intracellular calcium concentration in TRPV1-expressing cells (Caterina et al., 1997). TRP channels are widely expressed in excitable and non-excitable cells in both vertebrates and invertebrates and appear to represent primary pathways for regulated calcium cell entry (O'Neil and Brown, 2003).

TRPV1 is highly expressed in sensory neurons, including the dorsal root ganglia and trigeminal ganglia, with moderate expression in other tissues such as pancreas, brain,

liver, bladder, kidney, bowel, and others (Szallasi et al., 2007). When the channel is readily activated by vanilloids such as capsaicin, it appears to do so by direct binding of the ligand to an intracellular binding site on the channel (Lishko et al., 2007) (see figure 2.4). Other TRPV channels have been discovered, such as TRPV2, TRPV3, and TRPV4; and they have also been shown to be activated by heat, although it is thought they may have a much broader range of functions in addition to thermal sensation, including vascular function and inflammation (Baylie and Brayden, 2011, De Petrocellis et al., 2012).

Figure 2.4 The proposed structure of TRPV channels (O'Neil and Brown, 2003)



The channel is proposed to have 6 transmembrane domains (TM1-TM6), a pore loop (PL, responsible for channel permeability), a long NH2 terminal and COOH terminal, both existing in the cell's cytoplasm. The NH2 terminal has between 2 and 5 ankyrin repeat domains (ARD, commonly occurring protein structures in cells).

2.7.1.2 Mechanism of Action of Effects on Energy Expenditure

Capsaicinoids and capsinoids have been observed to cause increases in energy expenditure in both human (Lee et al., 2010b, Ludy and Mattes, 2011a) and animal

studies (Kawabata et al., 2009). It has been observed in several studies involving animal models that capsaicin treatment increased serum catecholamine levels.

Rats receiving capsaicin injections (intraperitoneally, 6mg/kg) responded in a manner similar to that of the metabolism of the catecholamine, epinephrine. Oxygen consumption increased upon injection, as did the respiratory quotient, and levels of serum glucose and immunoreactive insulin rapidly increased after administration of capsaicin. Also, liver glycogen rapidly decreased, in contrast to the serum glucose concentration which rapidly increased and the serum-free fatty acid level gradually increased. Alterations in energy metabolism which are all similar to those that occur in the metabolism of epinephrine (Kawada et al., 1986). This is supported by evidence in human trials, where capsaicinoids added to a meal significantly increased plasma epinephrine and norepinephrine levels compared with a control condition (Lim et al., 1997). In another experiment supplementation with propranolol (which inhibits the action of catecholamines (Atlas et al., 1974) abolished an increase in energy expenditure that was observed when subjects were given a capsaicinoid containing meal without propranolol (Yoshioka et al., 1995); thus providing evidence that an increase in energy expenditure is caused by the action of catecholamine secretion, as a result of capsaicinoid ingestion. These results suggest that the mechanism of action of capsaicinoids on the increase of energy metabolism is caused by a direct effect (as an agonist) on cells' catecholamine receptors or by the stimulation catecholamine secretion which in turn causes a beta-adrenergic response.

2.7.1.2.1 Effect of Brown Adipose Tissue

Evidence has also emerged that brown adipose tissue (BAT) may play an important role in capsaicinoid/capsinoid stimulation of energy expenditure. BAT evolved in mammals to dissipate large amounts of chemical energy as heat (Seale and Lazar, 2009). Brown fat cells possess large numbers of mitochondria that contain a unique protein called uncoupling protein 1 (UCP1). UCP1 functions to dissipate the proton motive force that is normally used to drive the synthesis of cellular ATP (Cannon and Nedergaard, 2004). As a consequence of UCP1 action, the energy in the mitochondria is released in the form of heat. BAT is a key thermogenic tissue in rodents and other small mammals, along with new-born humans that maintain core body temperature in

cold weather. The sensation of cold causes sympathetic nerves to release catecholamines in BAT that stimulate proliferation and heat production by brown fat cells (Lee et al., 2010a). Studies in rodents have also unequivocally demonstrated that BAT plays an essential role in energy balance and that its activity profoundly influences body weight (Seale et al., 2009). Though BAT persists as a distinct tissue in small mammals, the major deposit of BAT in new-born humans (between the shoulder blades) regresses shortly after birth (see figure 2.5). Other depots of BAT have been known to exist in adult humans for many decades, although levels vary greatly between individuals (Nedergaard et al., 2007). Age has been shown to be a key factor for BAT levels in humans, with research showing that cold-activated BAT was detected in about 55% of individuals in their 20s, but was less than 10% for those in their 50s and 60s (Yoneshiro et al., 2011).

Figure 2.5 Showing the results fluorodeoxyglucose in combination with computed tomography analysis of subjects with detectable and non-detectable levels of BAT (Saito and Yoneshiro, 2013).

Subject with detectable levels of BAT *Subject with non-detectable levels of BAT*

Besides exposure to cold, dietary stimuli can trigger the sympathetic nerve-mediated activation of BAT (Young et al., 1982, Nakamura and Morrison, 2007). Capsiate administered intragastrically to rats, resulted in a time- and dose-dependent increase in integrated BAT sympathetic nerve activity. This increase in sympathetic nerve activity was abolished when TRP channels were blocked and was inhibited by the removal of the gastrointestinal vagus nerve. The activation of sympathetic nerve activity was limited to BAT and did not occur in the heart or pancreas, suggesting BAT

may play an important role in stimulating the nervous system to increase energy expenditure and aid weight loss (Ono et al., 2011). This provided the first evidence that BAT may be playing an important role in increasing energy expenditure. This effect was replicated in a human trial (see section 2.7.3 for full details) which showed a significant increase in energy expenditure ($p < 0.05$) following capsinoid ingestion in the BAT positive group compared to placebo but a minimal increase in energy expenditure in the BAT negative group under the same conditions (Yoneshiro et al., 2012). Taken together these trials indicated that the presence of BAT may be necessary for capsaicinoids and capsinoids to produce an increase in energy expenditure. This would explain some of the variation in results seen across intervention trials, although further research is required to confirm this.

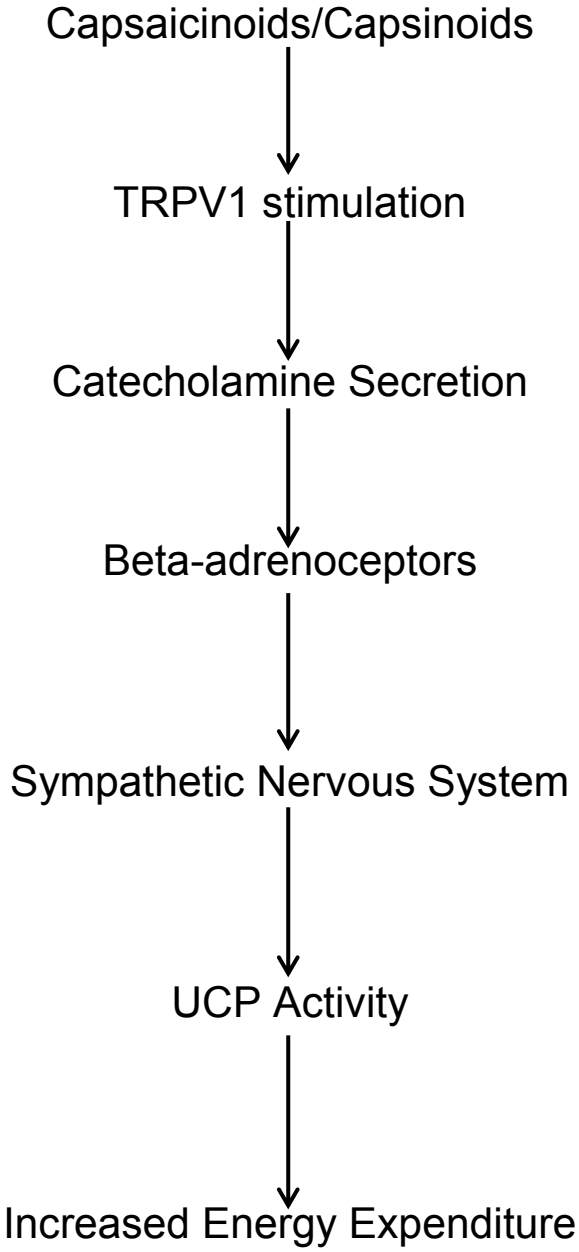
Interestingly results from another trial by the same research group suggested that six weeks of capsinoid supplementation in human participants (n=10) either increased the amount of BAT or increased the functioning of existing BAT, which in turn increases energy expenditure following supplementation (Yoneshiro et al., 2013). This may be due to recent findings that chronic sympathetic activation produces not only an increase in BAT cells but also a remarkable induction of UCP1-positive brown-like adipocytes in white adipose tissue (WAT). When this occurs, adipose cells have been termed ‘beige or brite’ cells, in mice and rats (Cinti, 2009). While BAT produces heat, WAT cells’ primary function is to store lipids. The existence of beige cells suggests that despite being structurally different adipose cells may be able to undergo a reversible physiological trans-differentiation and take on aspects of the other cell type’s functioning (Cinti, 2007).

A recent trial in which mice received capsaicin supplementation (2mg/kg) for three months found alterations in white and brown adipose tissue compared with the control group. Capsaicin supplementation increased gene expression associated with ‘browning’ of WAT (i.e. turning white adipose tissue into brown or ‘beige’ tissue) and increases expression of thermogenesis and mitochondrial biogenesis genes in already present BAT (Baboota et al., 2014). In addition to this it has been observed that BAT activity in humans is markedly increased during winter in individuals who showed undetectable activities in summer (Saito et al., 2009). Taken together this data suggests that human BAT identified by FDG-CT is largely composed of beige cells

and that its activity can be increased in response to appropriate sympathetic stimulation. This suggests that capsaicinoids and capsinoids, over time, may increase energy expenditure (via BAT) and also increase the amount of BAT (or beige adipose tissue) in humans.

2.7.1.2.2 Overview of Mechanism of Action for Energy Expenditure

Figure 2.6 A diagram to show the proposed process of increased energy expenditure by capsaicinoids and capsinoids, adapted from (Hursel and Westerterp-Plantenga, 2010).



Capsaicinoids and capsinoids stimulate the TRPV1 receptors in cells, which leads to catecholamine secretion from the adrenal medulla (Watanabe et al., 1987). The release of catecholamines into the body is detected by cells via their adrenergic receptors, many cells possess these receptors, and the binding of a catecholamine to the receptor will generally stimulate the sympathetic nervous system.

This is thought to then lead to an effect on uncoupling proteins (UCPs). Cellular energy production takes place across the inner mitochondrial membrane (adenosine diphosphate is converted to adenosine triphosphate using a gradient of protons produced by the respiratory chain). If protons leak back, abolishing the proton gradient, heat is created (a process known as thermogenesis) instead of normal energy usage. This disruption of the connection between food breakdown and energy production is known as “uncoupling”, and could lead to an increase in energy expenditure (Berger et al., 1998).

Details of human intervention trials investigating the effect of capsaicinoids on energy expenditure can be found in table 2.5 below. Six of the 10 trials using capsaicinoids and 8 of the 10 trials using capsinoids found a significant increase on energy expenditure following an intervention, with figures suggesting an increase between 41.8kJ – 209.2kJ (10kcal – 50kcal). The majority of trials involved a single dose of the intervention so it is not clear from these results if the effects would last over the longer term.

Table 2.5 Intervention trials evaluating the effects of capsaicinoids on energy expenditure

Author	Intervention Used	Dosage (mg)	Size (n)	Duration	Study Type	Key Findings
Henry & Emery 1986	Fresh Chilli	Not stated	12	Single meal	Randomised control	Chilli substantially increased RMR in all subjects
Yoshioka et al 1995	Dried chilli powder	30	8	Single meal	Randomised control	Increase in energy expenditure (EE) for 30 minutes (significance not stated).
Lim et al 1997	Dried chilli powder	Not Stated	8	Single meal	Randomised control	Increase in EE of 10% for 30 minutes (not significant)
Yoshioka et al 1998	Dried chilli powder	30	14	4 meals	Randomised crossover	Increase in EE ($p < 0.05$) for 180 minutes.
Matsumoto et al 2000	Curry sauce with chilli	3	16	Single meal	Randomised control	Significant increase seen in lean ($p <$

						0.01) but not obese group.
Lejeune et al 2003	Capsaicin supplement	135/day	91	4 months	Randomised placebo controlled	Significant increase in resting EE ($p < 0.005$).
Smeets & Westerterp-Platenga 2009	Capsaicinoid containing meal	5	30	Single meal	Randomised crossover	No effect on EE detected.
Ludy & Mattes 2011	Dried chilli powder or capsule	Variable	25	6 meals	Randomised crossover	Significant increase in EE at higher dose ($p = 0.013$).
Janssens et al 2013	Dried chilli powder	7.68/day	15	4 x 36 hr testing periods	Randomised crossover	Diet induced decrease in EE attenuated ($p=0.02$)
Smeets et al 2013	Capsaicin supplement	2.56	28	36 hr testing period	Randomised crossover	Increase in EE when consuming a calorie restricted diet ($p < 0.05$).

Table 2.6 Intervention trials evaluating the effects of capsinoids on energy expenditure

Author	Intervention Used	Dosage (mg)	Size (n)	Duration	Study Type	Key Findings
Ohnuki et al 2001	CH-19 Sweet pepper	0.03-0.1 /kg	11	Single meal	Randomised control	Increases in body temperature ($p < 0.01$) and oxygen consumption ($p < 0.03$).
Kawabata et al 2006	CH-19 Sweet pepper	1/kg	7	2 weeks	Randomised control	Decrease in body weight ($p < 0.05$)
Inoue et al 2007	Capsinoid supplement	3 or 10	44	4 weeks	Randomised placebo control	Dose responsive increase in EE significant only in those with BMI > 24.
Snitker et al 2009	Capsinoid supplement	6	80	12 weeks	Randomised placebo control	EE 54 kcal/d higher in treatment group ($p = 0.19$).
Galgani & Ravussin 2010	Dihydrocapsiate supplement	3 & 9	78	4 weeks	Randomised placebo control	Increased in EE ($p = 0.04$), around 50 kcal/d.
Galgani et al 2010	Capsinoid supplement	1, 3, 6 & 12	13	5 days	Randomised placebo control	No effect on energy expenditure found.
Josse et al 2010	Capsinoid supplement	10	12	Single meal	Randomised placebo control	Increases in EE ($p < 0.05$).
Lee et al 2010	Dihydrocapsiate supplement	3 & 9	33	4 weeks	Randomised placebo control	Increase in EE at high dosage ($p < 0.05$). Dose dependant effect observed.
Yoneshiro et al 2012	Capsinoid supplement	9	18	2 days	Randomised placebo control	Significant increase in energy expenditure in those with Brown Adipose Tissue (BAT)
Yoneshiro et al 2013	Capsinoid supplement	Not stated	10	6 weeks	Randomised placebo control	Significant increase in energy expenditure ($p < 0.01$), increase in BAT activity

2.7.1.3 Mechanism of Action of Effects on Lipid Oxidation/Adipose Tissue Accumulation

It has been observed in a number of human intervention trials that capsaicinoid and capsinoid supplementation taken over a number of weeks reduces body adiposity even if body weight is not reduced (Lejeune et al., 2003, Snitker et al., 2009). In addition to this, studies that have monitored expired breathing gases have noticed an increase in lipid oxidation after consumption of capsaicinoids (Janssens et al., 2013, Lee et al., 2010b).

Through secretion of adipokines (cell signalling proteins, released by adipose tissue) into the blood, adipose tissue plays a central role in development of obesity and metabolic disorders such as insulin resistance, diabetes, hyperlipidemia, hypertension and cardiovascular disease (Bastard et al., 2006, Palomo et al., 2006). In particular, white adipose tissue (WAT) which functions as an energy storage organ through formation of triacylglycerol and release of fatty acids into the bloodstream during a shortage of energy has a large influence (Trayhurn and Beattie, 2001). As a result of over-nutrition, excess WAT builds up and plays a major role in obesity and obesity-related disorders through dysregulation of normal metabolic functioning (Ouchi et al., 2011, Galic et al., 2010). Therefore, inhibition of excess WAT can be an efficient strategy for prevention of obesity and metabolic disorders (Kusminski and Scherer, 2012). Several potential mechanisms of action have been detected in research trials that may account for the observation that capsaicinoids could be inhibiting the build-up and function of adipose tissue.

2.7.1.3.1 Effect of the Sympathetic Nervous System

Animal studies have shown the sympathetic nervous system (SNS) to have several effects on white adipose tissue, including mobilisation of lipid stores. The SNS is the principal initiator of lipolysis (Bartness et al., 2001), which involves the hydrolysis of triglycerides into glycerol and free fatty acids. Once broken down these free fatty acids are released and transported through the blood stream (via lipoproteins) and are available for cellular uptake throughout the body (Wakil, 2012). Therefore capsaicinoid and capsinoid stimulation of the SNS may account for some of the increased level of lipid oxidation observed in human subjects.

In addition to initiating lipolysis, stimulation of the sympathetic nervous system has been demonstrated to effect adipocyte proliferation (Bartness and Song, 2007). Norepinephrine added to white pre-adipocyte cells (in vitro) was found to inhibit their normal proliferation. It has also been observed that surgical denervation of WAT produces pronounced (approximately 2-fold) increases in fat cell number with little change in fat cell size in rats (Cousin et al., 1993). In addition, obesity typically is associated with decreases in SNS activity (Bray, 1989), it may be that these two characteristics of obesity are related to one another; indeed, it has been hypothesized that decreases in SNS activity trigger increased adipocyte proliferation (Bartness et al., 2005). So stimulation of the SNS by capsaicinoids and capsinoids may also reduce the proliferation of adipose cells. It should however be noted that much of the research here has not involved capsaicinoids to stimulate SNS; therefore it is not definitive from this research that these capsaicinoids are affecting lipolysis and adipocyte proliferation in this way.

2.7.1.3.2 Effect of TRPV1 Channel

TRPV1 channels have been detected in pre-adipocytes cells (pre-cursors to adipocytes which have not yet fully differentiated) found in both human and mouse visceral adipose tissue (Zhang et al., 2007). When researchers induced the process of adipogenesis (creation of adipocytes from pre-adipocytes) in vitro, capsaicin dose-dependently induced calcium influx into the cells and prevented the adipogenesis. In the same study the authors carried out in vivo experiments where mice fed a high fat diet in the presence of capsaicin exhibited lower body weight and higher TRPV1 expression compared with mice on a high fat diet alone. In TRPV1 knock-out mice there was no significant difference in body weight between mice on a high fat diet with or without capsaicin. (Zhang et al., 2007). The results of this trial suggest that capsaicin may prevent the formation of adipocytes causing a decrease adipose tissue.

2.7.1.3.3 Effect on Cellular Enzyme and Protein Expression

A trial investigating the inhibition of lipid accumulation in adipocytes due to capsaicinoids suggests they may affect adipose enzyme expression. Capsaicin significantly decreased the amount of intracellular triglycerides and inhibited expression of an enzyme that plays a major role in lipid biosynthesis from

carbohydrates (glycerol-3-phosphate dehydrogenase) and an enzyme that regulates fatty acid storage activity in adipocytes (peroxisome proliferator-activated receptors gamma) (Hsu and Yen, 2007). This investigation suggests that capsaicin could reduce lipid storage in adipocytes, potentially reducing adipose tissue size.

Research in rats treated with capsaicin (10 mg/kg) along with a high-fat diet found that in comparison with control rats, body weight decreased by 8%. The researchers carried out a protein mapping procedure of the rats white adipose tissue, which revealed significant alterations to a number of proteins. 10 were significantly up-regulated and 10 were remarkably down-regulated in rats treated with a capsaicin supplemented diet compared to the control rats; most of the identified proteins are associated with lipid metabolism and redox regulation (Joo et al., 2010). These results suggest that thermogenesis and lipid metabolism related proteins were markedly altered in WAT upon supplementation with capsaicin, and demonstrate more of the effects that capsaicinoids and capsinoids may be exerting at a cellular level on adipose tissue.

2.7.1.3.4 Effect on Nerve Stimulation

As capsaicinoids and capsinoids are potent nerve agonists, a number of researchers have considered their impact on nerve signals and whether this could affect body fat accumulation and distribution. Capsaicin (a dose of 5 mg/kg) administered straight into the body cavity was found to desensitise local afferent vagal nerve endings for approximately 3 weeks in rats. Following this desensitization, the rats deprived of food for 120 h lost significantly more weight than the controls ($p < 0.05$), suggesting the blockade of such nerve signals might enhance loss of body weight upon fasting (Garami et al., 2010). The impact of disrupting vagal signalling by surgical restriction (SR) or de-sensitisation with capsaicin (DS) on weight gain and fat content was assessed in diet-induced obese rats. Rats underwent vagal SR or DS with capsaicin, or a sham procedure. Animals were maintained for 11 months on a high-calorie, Western style diet; SR rats weighed 19 % less ($P = 0.003$) and DS rats 7 % less ($P = 0.19$) than the control group; SR and DS animals had a 52 % ($P < 0.0001$) and an 18 % reduction ($P = 0.039$) in visceral abdominal fat, respectively. Disrupting vagal signalling by SR led to significant reductions in both diet-induced weight gain and

visceral abdominal fat deposition; and vagal DS led to more modest, but clinically significant reductions in weight and visceral abdominal fat (Stearns et al., 2012).

Also investigated have been intestinal mucosa afferent nerve terminals which can mediate post-prandial changes in intestinal and visceral adipose tissue blood flow (Leung, 2008). Capsaicinoids and capsinoids are a potent afferent nerve stimulators and functional impairment of these sensory sites has been shown to retard body weight gain in rats (Leung et al., 2007), in addition to this intestinal mucosal afferent nerve function may be necessary for the physiologic accumulation of fat in visceral adipose tissue sites (Leung, 2008). Evidence from these trials suggests capsaicinoids impact on nerve cells, particularly along the vagus nerve and the intestinal mucosa afferent mechanism, may regulate adipose tissue distribution and prove to be of health benefit when ingested orally.

Details of human intervention trials investigating the effects of capsaicinoids on energy expenditure can be found in table 2.8 and the effect of capsinoids in table 2.9 below. Four of five capsaicinoid trials and four of six capsinoid trials found a significant increase in lipid oxidation following an intervention. Results suggest an increase in lipid oxidation of around 20% in the period following a capsaicinoid/capsinoid intervention. The majority of the trials involved a single intervention so it is not clear from these trials whether the effect would be repeated over the longer term.

Table 2.8 Intervention trials evaluating the effects of capsaicinoids on lipid oxidation

Author	Intervention Used	Dosage (mg/day)	Size (n)	Duration	Study Type	Key Findings
Yoshioka et al 1995	Capsaicin rich red pepper	30	8	Single meal	Randomised control	Decrease in lipid oxidation (significance not stated).
Yoshioka et al 1998	Capsaicin rich red pepper	30	14	4 meals	Randomised crossover	Increase in lipid oxidation ($p < 0.05$) for 180 mins.
Lejeune et al 2003	Capsaicin supplement	135	91	4 months	Randomised placebo controlled	Lipid oxidation increase ($p < 0.05$), No significant effect on weight.
Shin and Moritani 2007	Capsaicin capsule	150	10	Single meal	Randomised crossover	Increase in lipid oxidation ($p = 0.05$) during exercise
Janssens et al 2013	Dried chilli powder	7.68/day	15	4 x 36 hr testing periods	Randomised crossover	Increase in lipid oxidation ($p = 0.03$)

Table 2.9 Intervention trials evaluating the effects of capsinoids on lipid oxidation

Author	Intervention Used	Dosage (mg)	Size (n)	Duration	Study Type	Key Findings
Inoue et al 2007	Capsinoid supplement	3 & 10	44	4 weeks	Randomised placebo control	Increased lipid oxidation ($p < 0.05$), significance only reached in group with BMI > 25.
Snitker et al 2009	Capsinoid supplement	6	80	12 weeks	Randomised placebo control	Abdominal fat decreased ($p = 0.049$). Lipid oxidation increased ($p = 0.06$).
Galgani & Ravussin 2010	Dihydrocapsiate supplement	3 & 9	78	4 weeks	Randomised placebo control	No changes in lipid oxidation or body fat detected.
Galgani et al 2010	Capsinoid supplement	1, 3, 6 & 12	13	5 days	Randomised placebo control	No effect on lipid oxidation found.
Josse et al 2010	Capsinoid supplement	10	12	Single meal	Randomised placebo control	Increases lipid oxidation, decrease in blood free fatty acids and glycerol (all $p < 0.05$).
Lee et al 2010	Dihydrocapsiate supplement	3 & 9	33	4 weeks	Randomised placebo control	Increase in fat oxidation ($p < 0.05$).

2.7.1.4 Effects on Appetite and Energy Intake

It has been observed in a number of human intervention trials that an effect on appetite and energy intake may occur when the diet is supplemented with capsaicinoids (Janssens et al., 2014, Ludy and Mattes, 2011a) or capsinoids (Reinbach et al., 2009). In addition to this, a meta-analysis (carried out as part of this research, see chapter 3) suggested that consuming capsaicinoids prior to a meal could reduce energy intake by around 79 kcal (310 kJ) (Whiting et al., 2014). Research suggests supplementation may reduce appetite and desire to eat as well as number of calories eaten by reducing the actual amount of food eaten or by changing food choice to a preference for lower calorie food (increased intake carbohydrate-rich food at the expense of fat-rich foods) (Ludy et al., 2012). A number of possible mechanisms of action have been suggested for this observation and they will be discussed in this section.

2.7.1.4.1 Effect of Intestinal Hormones

In human trials it has been observed that capsaicin could affect energy intake without oral stimulation (Westerterp-Plantenga et al., 2005). Therefore it has been hypothesised that capsaicinoids and capsinoids may exert an influence on appetite via stimulation sites in the intestinal tract; evidence has emerged from a number of

trials to support this. In animals, capsaicin has been used to de-sensitise gut neurons (especially along the vagal nerve), which has altered the function of a number of hormones important to appetite control and gastric emptying, including glucagon-like peptide-1(GLP-1) (Ahrén, 2004), ghrelin (Fukuda et al., 2004) and peptide YY (PYY) (Fu-Cheng et al., 1997). Research suggests gastric emptying it is a key mediator of hunger, satiation and satiety (Janssens et al., 2011). Capsaicin may influence gastric motility and was found to significantly increase the gastric emptying rate in a study involving 10 human subjects, when given intragastrically (Debreceni et al., 1999) This in turn may influence the rate at which hormones such as GLP-1, ghrelin and PYY are released, as the speed at which chyme is released into the intestinal passage is an important factor in stimulating cells to release these hormones (Berthoud, 2008).

GLP-1 is a hormone released by cells distributed throughout the small intestine as a result of the presence of nutrients (Sathananthan et al., 2013), it has been shown to decrease food intake by increasing satiety signals in the brain (Holst, 2007). Ghrelin, also produced by intestinal cells, that is a strong stimulator of appetite (Van Der Lely et al., 2004). Ghrelin secretion is stimulated when the stomach is empty, and halted when the stomach is stretched (Anderson et al., 2005). It acts on hypothalamic brain cells both to increase hunger, and to increase gastric acid secretion and gastrointestinal motility to prepare the body for food intake (Schwartz et al., 2000). Ghrelin also plays an important role in regulating reward perception in this area of the brain through its interaction with dopamine and acetylcholine (Burger and Berner, 2014). PYY is another intestinal hormone that can have an influence on food intake and gastric emptying and is usually secreted alongside GLP-1 (Cox et al., 2010). Secretion is mainly stimulated by the presence of food in the digestive tract and higher circulating levels result in decreased appetite and energy intake, along with reduced gastric motility (Nguyen et al., 2011a).

Changes in these hormones could have a potentially strong impact on appetite and energy intake and all three have been measured following capsaicinoid intake in 30 human subjects. For 45 minutes after consumption of the capsaicin containing meal plasma GLP-1 response was significantly higher compared to the control meal ($p < 0.05$) and ghrelin response tended to be lower, although not significantly ($p < 0.07$); PYY responses were not different between the two groups (Smeets and Westerterp-

Plantenga, 2009). These results suggest a potential mechanism of action for capsaicinoids influencing appetite and energy intake (as observed in other trials) by affecting the influence of intestinally derived satiety hormones. Possibly this is caused by increased gastric motility, thereby increasing the speed of exposure of nutrients to hormone releasing cells in the intestinal passage.

2.7.1.4.2 Effect of the Sympathetic Nervous System

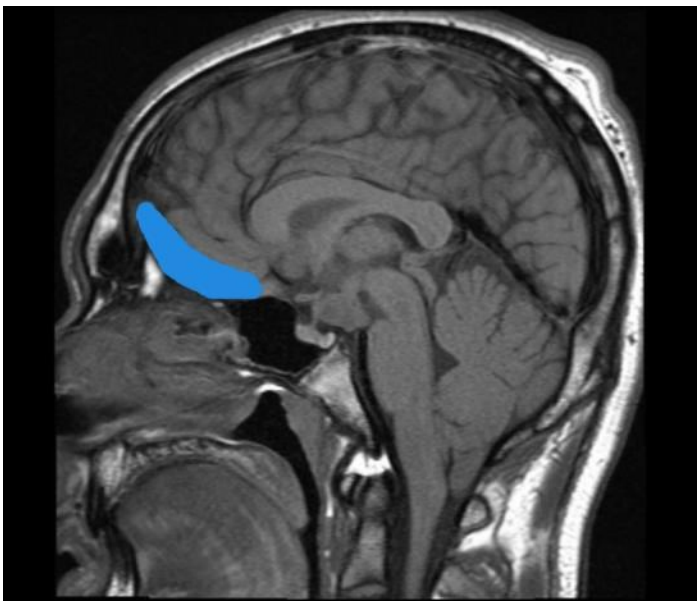
Capsaicinoids and capsinoid may also be influencing appetite through their action on TRPV1 receptors and the resulting stimulation of the sympathetic nervous system (SNS). As mentioned earlier, it is generally accepted that capsaicinoids and capsinoids stimulate the SNS, causing the release of catecholamines (Hursel and Westerterp-Plantenga, 2010). Activation of certain adrenoceptors, caused by norepinephrine has been shown to decrease food intake (Wellman, 2000), notably beta-receptors (β2 and β3), which research suggests are stimulated as a result of capsaicinoid and capsinoid intake. Indeed the release of catecholamines, has been the mechanism of action for some appetite suppressant drugs such as Sibutramine (Adan et al., 2008). It is thought the release of catecholamines (particularly norepinephrine) has a signalling effect on the hypothalamus, a region of the brain that plays a crucial role in appetite control (Suzuki et al., 2010). Therefore the release of catecholamines and sympathetic nervous system stimulation is another potential mechanism of action for capsaicinoids and capsinoids influencing appetite.

2.7.1.4.3 Effect on the Brain

It was found in one investigation into capsaicinoids' effect on energy intake that supplementation reduced calorie intake not by reducing the amount of food eaten, but rather by a shift in preference for carbohydrate-rich food over fat-rich food (Westerterp-Plantenga et al., 2005). Therefore capsaicinoids may be affecting areas of the brain involved in food choice and reward through stimulation of neurons in the mouth. This is possible as capsaicinoids are a potent stimulator of nociceptors in the mouth, which are responsible for detecting temperature and pain (Woolf and Ma, 2007) and would likely to have been important in detecting harmful food substances throughout human evolution (Julius and Basbaum, 2001).

The orbitofrontal cortex is an important area of the brain for the convergence of representations of the taste, smell, sight, and mouth feel of food (see figure 2.7), and this convergence allows the sensory properties of each food to be represented and defined in detail (Rolls, 2004b). This is also the region where a short-term, sensory-specific control of appetite and eating is implemented (Rolls, 2004a). Capsaicin has been shown to be a strong activator of neurons in this part of the brain, via oral stimulation in primates (Kadohisa et al., 2004). The orbitofrontal cortex also seems to be important in signalling the expected rewards or punishments of a particular action (Rolls and Grabenhorst, 2008) and may also go some way to explaining why different people experience such different reactions to capsaicinoid exposure. The impact of capsaicinoids on the orbitofrontal cortex provides another potential mechanism of action of the observed effect of reducing energy intake by affecting food choice. It should be noted that research and understanding in this area is still developing and much of the research has been with non-human primate brains, opening the possibility that effects may be different in humans.

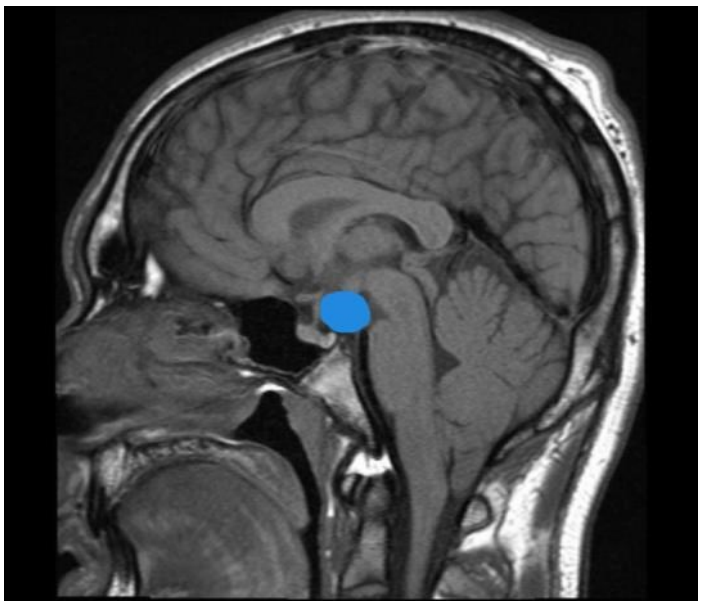
Figure 2.7 A MRI brain scan showing the location of the orbitofrontal cortex.



Another area of the brain that plays a critical role in energy balance, affecting energy intake and food reward mechanisms, is the hypothalamus (Dietrich and Horvath, 2013). Located in at the top of the human brain stem (see figure 2.8), and as well as producing hormones, is the site in the brain affected by intestinally released satiety

hormones such as GLP-1, ghrelin and PYY (Smith and Thorner, 2012). Studies have suggested the presence of TRPV1 channels in different regions of the hypothalamus (Menigoz and Boudes, 2011) and that TRPV1 containing neurons are co-expressed with multiple neuropeptides with a potential role in satiety (Bishnoi et al., 2011). Recent research in mice has found that capsaicin increased expression of TRPV1 genes and increased the expression of potentially appetite suppressing genes in the hypothalamus compared to control mice (Baboota et al., 2014). Hinting that capsaicin may be effecting the hypothalamus to reduce energy intake over time. However, the hypothalamus' impact of energy intake is subject to the control of numerous neuropeptides (Zhang et al., 2011) and further research is required to confirm these early findings.

Figure 2.8 A MRI scan showing the location of the hypothalamus in a human brain.



Details of human intervention trial investigating the effects of capsaicinoids on energy intake can be found in table 2.11 below. Four of the 10 trials found a significant reduction in energy intake following a capsaicinoid intervention, with another of the trials finding a significant effect on circulating intestinal hormones. The majority of trials involved a single intervention, so it is not clear from these trials whether any potential effect would last over the longer term.

Table 2.10 Intervention trials evaluating the effects of capsaicinoids on energy intake

Author	Intervention used	Dose (mg)	Size (n)	Duration	Study Type	Key Finding
Yoshioka et al 1999	Meal with dried chilli	30	13	Single Meal	Randomised placebo control	Non-significant reduction in energy intake (EI)
Yoshioka et al 1999	Appetiser with dried chilli	18	10	2 Meals	Randomised placebo control	Significant reduction in EI ($p<0.05$)
Yoshioka et al 2004	Chilli soup or capsules	0.2, 3	16	Single Meal	Randomised double blind placebo control	Non-significant reduction in EI
Westerterp-Platenga et al 2005	Juice with chilli or chilli capsules	2.25	24	4 Days	Randomised double blind placebo control crossover	Significant reduction in EI ($p<0.05$), stronger effect with oral exposure
Ahuja et al 2007	Preserved chilli	30/day	36	8 weeks	Randomised controlled Crossover	No effect on EI
Reinbach et al 2009	Chilli capsules	2.5	27	6 meals	Randomised placebo control crossover	Significant reduction in EI during over-feeding ($p < 0.05$)
Smeets et al 2009	Meal with dried chilli	5	30	Single meal	Randomised placebo control crossover	Affected gastrointestinal hormones may reduce appetite
Reinbach et al 2010	Appetiser with chilli	0.4	40	Single Meal	Randomised placebo control crossover	No effect on EI
Ludy and Mates 2011	Chilli capsules	3.6	25	6 meals	Randomised double blind placebo control crossover	Significant reduction in EI ($p<0.05$), observed only in non-chilli eaters
Janssens et al 2014	Chilli capsules	2.56	15	4 x 36 hr testing periods	Randomised placebo control crossover	Capsaicin tended to reduce ad libitum energy intake ($p=0.07$)

2.7.1.6 Summary of Mechanism of Action for the Effects of Capsaicinoids and Capsinoids on Weight Loss and Weight Management

In conclusion, there are a number of ways in capsaicinoids and capsinoids ingestion may be influencing the body in a beneficial way to aid weight loss or maintenance. Perhaps the most studied and well-established of these is the pathway through which energy expenditure is increased, via stimulation of the TRPV1 receptor and the resulting effect on catecholamine release signalling to adrenoceptors, which stimulate the SNS and cause uncoupling protein regulation resulting in enhanced energy expenditure (through thermogenesis), with BAT potentially playing a crucial role in this process. The effect is likely to be relatively small however and it may be that an increased in lipid oxidation, resulting in lower adipose tissue levels, is a more significant health benefit of ingestion of capsaicinoids and capsinoids. A number of potential mechanisms of this were outlined, namely SNS stimulation/catecholamine

release, cellular TRPV1 stimulation, an effect on adipose tissue enzymes and protein expression and finally neuron stimulation. Less well established has been capsaicinoids potential effect on energy intake, which may be reduced following ingestion. This may be caused by capsaicinoids affecting intestinally derived satiety hormones, influencing certain areas of the brain, SNS stimulation, and increases in lipid oxidation. The effect on energy intake are less well established from intervention trial and therefore further investigations into these effects may be warranted.

2.7.2 Biochemistry

Studies in animals and humans show that topical and oral administration of capsaicinoids produce moderate circulating concentrations and high local concentrations, depending upon the dose and route of delivery. Using a capsaicin skin patch (dose 10mg/kg) with 1–1.5 hours of treatment time in humans, produced peak plasma concentrations of 0.5–18 ng/mL in the roughly 30% of patients where quantifiable capsaicin levels occurred; capsaicin was cleared in most patients within 4.5 hours (Babbar et al., 2009). Orally administered capsaicinoids are also efficiently (85–95% total dose) absorbed from the gastrointestinal lumen, with minor metabolism occurring in the intestine (mainly hydrolysis), but near complete breakdown in the liver (Suresh and Srinivasan, 2007). In one study, a 5 g oral dose of chilli (containing 26.6 mg capsaicin) in 12 humans yielded peak plasma concentrations of capsaicin of 2.5 ng/mL (Weerapan, 2009), with the author suggesting that the relatively low maximum concentration and quick metabolism were the result of extensive metabolic breakdown, consistent with prior studies (Donnerer et al., 1990, Kawada et al., 1984). Researchers investigating the metabolism of dihydrocapsaicin (a capsaicinoid compound), found that within 48 hours of oral administration (20 mg/kg body weight) to male adult rats, unchanged dihydrocapsaicin and eight of its metabolites (dihydrocapsaicin (8.7% of the total dose), vanillylamine (4.7%), vanillin (9.6%), vanillyl alcohol (37.6%) and vanillic acid (19.2%)) were identified in urine as free forms and/or their glucuronides. The authors concluded that these results provide strong support that capsaicin and its analogues when supplemented to the diet are well absorbed into blood (Kawada et al., 1984). In another study, oral capsaicin consumption in rats (30 mg/kg; approximately five times the average daily intake of capsaicinoids by individuals in India, a country with a high average capsaicin intake), demonstrated

94% absorption from the intestine with a peak serum concentration of 6.2 μM (Suresh and Srinivasan, 2010). In this same study, the authors investigated capsaicin concentrations in various organ tissues, finding substantially higher amounts, in the liver, kidneys and intestines.

Researchers have found capsaicinoids are highly responsive to biotransformation by P450 enzymes, which are located either in the inner membrane of mitochondria or in the endoplasmic reticulum of cells and are found in most tissues in the body. These enzymes converted capsaicinoids (capsaicin and nonivamide) into reactive, free radical intermediates, which the authors noted may partially explain conflicting reports related to the cytotoxic, pro-carcinogenic, and chemo-protective effects of capsaicinoids in different cells and organ systems (Reilly et al., 2012). The full biological implications of capsaicinoid bio-chemical metabolism remains unknown, with future studies needed to evaluate capsaicinoids' full effects as pharmaceutical, cytotoxic and chemo-preventive agents, although the research suggests it is well absorbed into the body from oral consumption.

Chapter 3 - Meta-Analysis of Capsaicinoids Effect on Energy Intake

3.1 Introduction

Research indicates that capsaicinoids may have a beneficial effect on weight management outcomes by reducing energy intake following ingestion. Ten small intervention studies have been carried out to investigate this hypothesis. However, results have been contradictory, with trials observing a statistically significant effect on energy intake in some instances (Westerterp-Plantenga et al., 2005) and no effect in others (Smeets and Westerterp-Plantenga, 2009). In order to provide further evidence for this hypothesis, a meta-analysis of intervention trials investigating the effect of capsaicinoid intake on energy intake was conducted herein. Results showed that capsaicinoid intake prior to a meal (in the form of supplement or dried chilli) reduced *ad libitum* energy intake by 251kJ (60kcal) $p < 0.001$ during the following meal. Results, however should be viewed with some caution as analysis indicates there was publication bias (p=0.007) The following chapter details the meta-analysis methodology, results and discusses how the result furthers our understanding of the potential use of capsaicinoids as a weight management aid.

3.2 Methods

3.2.1 Identification of Relevant Studies

Studies were identified by searching Web of Knowledge and PubMed search engines. No language restrictions were applied. The initial search was performed on 27th April 2012 and updated on 27th May 2016. The following search terms were used: 'capsaicin' combined (using Boolean 'AND') with 'energy intake' and 'appetite', in turn. Human, randomised, intervention trials, using 'healthy' volunteers, compared to themselves or to a matched control group were identified and included. 'Healthy' referred to participants free of disease, but included overweight and obese participants. Trials were initially selected based on their abstracts. Full content was then reviewed to determine inclusion in the final sample. References lists cited in the selected studies were also searched to find additional studies for inclusion.

Duplicate manuscripts, studies that did not investigate capsaicinoids' effect on energy intake and studies where the intervention included other bioactive ingredients (such as green tea and caffeine) were removed. Studies using multiple bioactive ingredients were also removed as it would not be clear how much of the observed effect was due to capsaicinoids. The remaining studies investigated the role of capsaicinoids on weight management in relation to their effect on energy intake, hunger and satiety measures (usually recorded on a 1-10 scale) and intestinal hormone levels. The most frequently used methodology was to measure *ad libitum* energy intake after capsaicinoid intake, therefore this was used in the main analysis. Studies using multiple bioactive ingredients were removed as it would not be clear how much of the observed effect was due to capsaicinoids. The remaining studies were reviewed to establish an appropriate effect size that could be extracted and analysed.

3.2.2 Data Extraction

Data extracted from the papers included: participant number, gender, age, BMI and ethnicity; study type, dosage, the intervention used and study duration (see tables 3.1 and 3.2 for further details). The study's authors and year of publication were also recorded. Effect sizes were then extracted for analysis, including mean energy intakes (in kJ) for control and intervention groups, along with standard deviations.

3.2.3 Statistical Analysis

The combined effect size was calculated using the random effects model and a forest plot produced along with the I^2 statistic to assess heterogeneity. Sub-group analysis was performed assessing differences according to participant ethnicity, age and BMI along with study size, dosage, length and type of intervention. Finally, publication bias was assessed by calculating Egger's regression statistic and producing a trim and fill plot. All statistical analyses were performed using MIX meta-analysis software version 2.0 (Bax et al., 2006).

3.3 Results

A total of 41 clinical trials were identified from the database searches. Twenty-eight trials were excluded for being duplicates (the same studies found on multiple searches) and a further seven were excluded for methodical reasons; five of these did not feature investigations examining energy intake and two used intervention featuring multiple bio-active ingredients (not just capsaicinoids), leaving a total of 6 trials (see figure 3.1). Three more studies were identified from searching the references of the trials initially found in the search. Leaving a final total of 9 studies (with 193 participants) included in the meta-analysis (see table 3.1 for details of study participants).

Figure 3.1 Literature search and inclusion/exclusion of trials

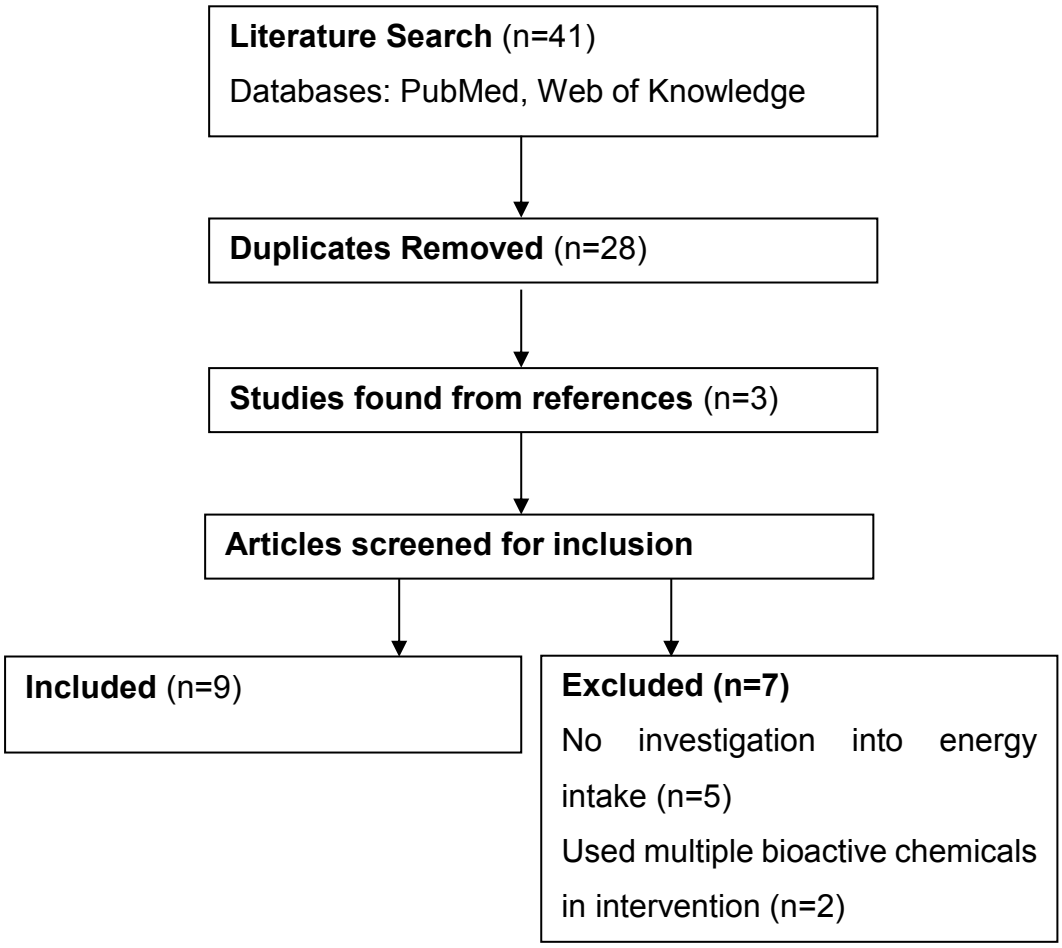


Table 3.1 Key characteristics of participants in the intervention studies

Trial	Gender	Ethnicity	BMI	Age
Yoshioka *et al* 1999(a)	Female	Asian	21.9	26
Yoshioka *et al* 1999(b)	Male	Caucasian	23.7	33
Yoshioka *et al* 2004	Male	Asian	25.6	22
Westerterp-Platenga *et al* 2005	Mixed	Caucasian	25.0	35
Ahuja *et al* 2007	Mixed	Mixed	26.4	46
Reinbach *et al* 2009	Mixed	Caucasian	22.2	29.6
Reinbach *et al* 2010	Male and Female Intervention groups	Caucasian	24.6	22.4
Ludy and Mates 2011	Mixed	Mixed	23.0	22.6
Janssens *et al* 2014	Mixed	Caucasian	23.3	29.7

There were no issues with adherence to the required diet reported in any of the studies. This may be due to their relatively short nature and the fact most of the meals were supplied by the researchers and consumed on site, with the exception of one study (Ahuja et al., 2007).

Nine of the eight selected studies followed this design: after randomisation, participants would consume a capsaicinoid intervention or placebo, followed by a test meal consumed at a research site. The participants would consume food ad libitum until full and the remaining food would then be weighed to provide an exact calculation of energy intake. The only exception to this (Ahuja et al., 2007), featured 4 weeks of chilli supplementation (added to meals by the participant at home) in addition to participants' normal diet, the trial featured a crossover design with 4 weeks of a control diet for comparison. Energy intake being assessed with a 4-day weighed food diary recorded in the final week of each dietary period.

Two trials attempted to stimulate periods over and under eating in participants; one featured 3 weeks of positive energy balance and 3 weeks of negative energy balance, prior to each test meal consumed at a research site (Reinbach et al., 2009). The other

trial attempted to simulate these conditions by including high fat or high carbohydrate meals as test meals (Yoshioka et al., 1999); with both investigations finding reductions in energy intake under both conditions. Another trial measured the effect of the hedonistic impact of capsaicinoids by using two different interventions, one capsules and one a spicy tomato juice (Westerterp-Plantenga et al., 2005). They observed a decrease in energy intakes under both conditions, but a stronger one with oral exposure to capsaicinoids. Dosages in the trials ranged from 0.4mg – 33mg and trial length varied from a single meal to a four week intervention (see table 3.2 for details).

Table 3.2 Key characteristics of the intervention trials included in the analysis

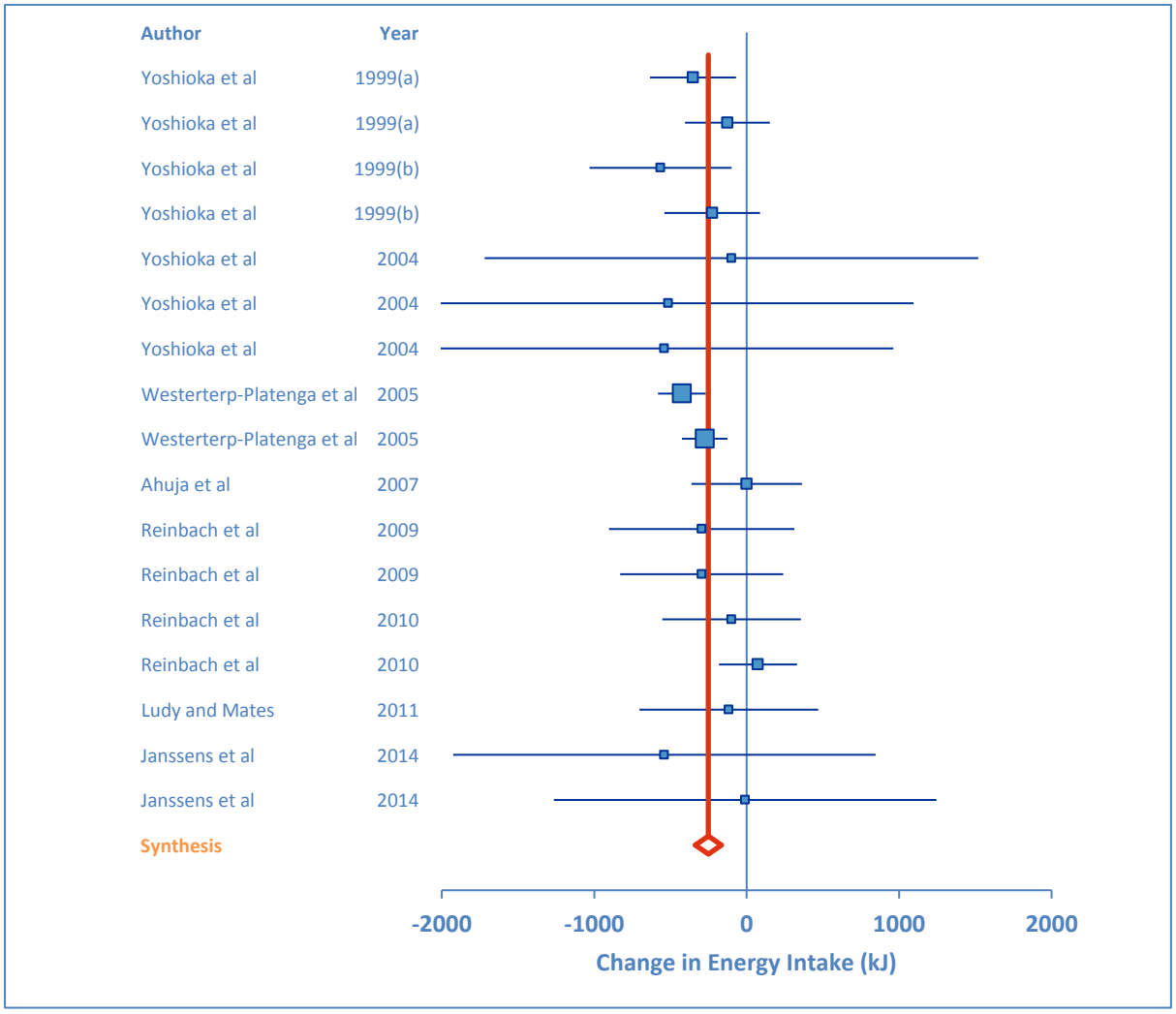
Trial	Size (n)	Dose (mg)	Intervention	Length	Key Finding
Yoshioka *et al* 1999(a)	13	30	Meal with dried chilli	Single Meal	Non-significant reduction in EI
Yoshioka *et al* 1999(b)	10	18	Appetiser with dried chilli	2 Meals	Significant reduction in EI ($p<0.05$)
Yoshioka *et al* 2004	16	0.2, 3	Chilli soup or capsules	Single Meal	Non-significant reduction in EI
Westerterp-Platenga *et al* 2005	24	2.25	Juice with chilli or chilli capsules	4 Days	Significant reduction in EI ($p<0.05$), stronger effect with oral exposure
Ahuja *et al* 2006	36	33/ Day	Preserved chilli	8 weeks	No effect on EI
Reinbach *et al* 2009	27	2.5	Chilli capsules	6 weeks*	Non-significant reduction in EI
Reinbach *et al* 2010	40	0.4	Appetiser with chilli	10 Meals*	No effect on EI
Ludy and Mates 2011	25	3.6	Chilli capsules	6 meals	Significant reduction in EI ($p<0.05$) in non-chilli eaters
Janssens *et al* 2014	15	2.56	Chilli capsules	36 hours	Non-significant reduction in EI

EI – energy intake

The meta-analysis assessed the change in energy intake following the consumption of a capsaicinoid intervention compared with a reference value. The combined effect size showed that consuming capsaicinoids caused a significant reduction in *ad libitum* energy intake of 251kJ (60kcal) per meal (see figure 3.2, for the forest plot), under the

random effects model, with a 95% confidence interval of 337 – 166kJ and $p < 0.001$. The analysis indicated low heterogeneity, $I^2 = 6.6\%$.

Figure 3.2 Forest plot showing the effect size of capsaicinoids v reference for all intervention studies.



An effect size of 0 represents no difference between interventions, an effect size < 0 represents a reduction in energy intake, >0 represents an increase in energy intake.

3.3.1 Sub-Group Analysis

To investigate whether different study and participant characteristics could influence the effect size, a number of subgroup analyses were performed. The variables analysed were: ethnicity, study size (n), intervention type (food or supplement), study length, dosage, average BMI and average participant age. P-values were also calculated comparing the *ad libitum* energy intake of the meal post intervention to the *ad libitum* energy intake of the control meal for each sub-group. Details of the analysis

are in table 3.3 below. Results suggest that ethnicity, study size, intervention type, study length, age and BMI had no effect on the overall effect size and therefore the effect of capsaicinoids on *ad libitum* energy intake in the present studies. However, the size of the dosage may have an impact on the effect of capsaicinoid intervention, with a dosage of 2mg or less seemingly having no effect on *ad libitum* energy intake.

Table 3.3 Effect sizes and comparative p-values for differing aspects of the intervention studies

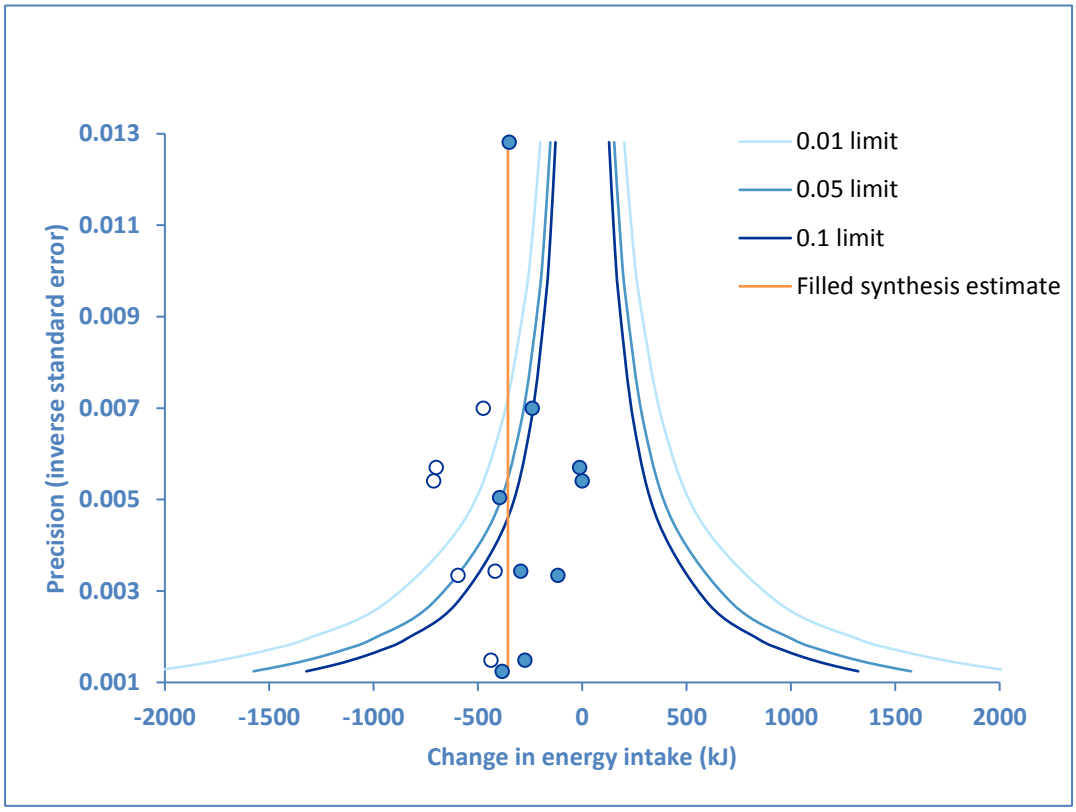
Comparison	Effect Size	p-value
Ethnicity	Asian -244kJ	0.01
	Caucasian -281kJ	<0.001
Study Size	≤20 participants -261kJ	<0.001
	>20 participants -256kJ	<0.001
Intervention Type	Food -254kJ	<0.001
	Supplement -270kJ	<0.001
Study Length	Single meal -184kJ	0.002
	Multiple meals -318kJ	<0.001
Dosage	≤2mg 29kJ	0.79
	>2mg -300kJ	<0.001
Age	≤30 -146kJ	0.03
	>30 -320kJ	<0.001
BMI	≤25 -180kJ	0.003
	>25 -319kJ	<0.001

A negative effect size shows a decrease in EI, a positive effect size shows an increase in EI following capsaicin ingestion.

3.3.2 Publication Bias

To investigate the possibility of publication bias, a trim and fill funnel plot was created (see figure 3.3 below). To ensure accurate analysis multiple results from single studies were averaged out so that only one effect size from each study was used in the analysis. Egger's regression was calculated as p=0.007, indicating that there was publication bias amongst the data found for this analysis. Results from the trim and fill plot suggest that publication bias has led to an underestimate of the effect size. Inclusion of predicted effect sizes for unpublished analyses, resulted in capsaicinoid ingestion causing a decrease in *ad libitum* energy intake of -356kJ and a 95% confidence interval of -447kJ to -265kJ.

Figure 3.3 Trim and fill plot detailing change in energy intake following correction for potential publication bias



Effect sizes of actual studies shown in blue, generated effect sizes shown in white

3.4 Discussion

The results suggest adding capsaicinoids to the diet could have a beneficial effect for weight management, by reducing energy intake. However, caution should be applied to this result due to the small size of the reduction and the short term nature of the trials involved. A calorie imbalance of 2100kJ (500kcal) per day is often suggested as ideal for weight loss (Stern et al., 2004), although smaller imbalances can also lead to weight loss and improved health outcomes (Hill, 2006). Therefore, a reduction of 251kJ per meal (95% confidence interval of 337 – 166kJ) repeated three times a day may have the potential to lead to beneficial weight loss. Furthermore, evidence of publication bias was identified which suggests that the estimate is an underestimate and may be 356kJ (95% confidence interval -447kJ to -265kJ.). The I^2 statistic was low at 6.6%, this suggest that only a small amount of the variance observed between the study is due to heterogeneity.

Analysis into publication bias suggested there is likely to be studies among the literature investigating capsaicinoids' effect on energy intake not included in this analysis, with Egger's regression was calculated as p=0.007. The trim and fill plot calculated to estimate the potential effect of these missing studies on the combined effect size suggests the true effect of capsaicinoids on energy intake may be larger than has been calculated in this analysis.

The results of the sub-groups analysis suggest that the dosage of capsaicinoids used is an important factor in producing a reduction in energy intake. The combined effect size of the lowest dosage trials (≤2mg of capsaicinoids) showed no reduction in energy intake following capsaicinoid ingestion, whereas the combined effect size of those trials with a dosage >2mg produced a statistically significant reduction in energy intake of 300kJ. Suggesting a dose above 2mg is necessary to produce an effect. It is not clear from these studies what the ideal dose of capsaicinoids to reduce energy intake would be as there were not enough results from the trials to produce an informative dose response curve. Doses varied from 0.4mg (Reinbach et al., 2010) to 33mg (Yoshioka et al., 1999).

All the other sub groups individually analysed, produced a statistically significant reduction in energy intake. Suggesting capsaicinoids still produced an effect on energy intake regardless of study participant's ethnicity, age, BMI, whether given in the form of supplements or chillies, whether given over a single meal or multiple meals and the number of participants in the study.

It has been suggested that the effects of capsaicinoids may be stronger among Asian populations (Hursel and Westerterp-Plantenga, 2010). The suggestion being that due to chillies being part of their traditional diet, some genetic adaptation may have occurred. The subgroup analysis did not however support this observation, with the effect being slightly larger in the Caucasian population (281kJ compared to 244kJ in Asian population). Although informative, this result should be viewed with some caution, as it involved only a small number of Asian participants (29 Japanese participants in 2 trials); therefore it is not possible to conclude that there was a different effect among different ethnicities. It should also be noted that the studies only featured

Asian and Caucasian participants, so no conclusions can be drawn about capsaicinoids effect on energy intake among other ethnicities.

There was little variance among the average BMIs of the study participants, ranging from 21.9 – 26.4kg/m^2, therefore it is difficult to draw any conclusions concerning whether capsaicinoids may affect energy intake in persons of normal weight range or overweight/obese range differently. In addition the majority of the study participants were young adults having an average participant age between 22-35 years, with one study the exception (Ahuja et al., 2007) having an age range between 22-70. Therefore capsaicinoids effect on energy intake among under-18s and older adults is not known from the present analysis.

None of the studies reported issues with compliance of the capsaicinoid intervention, this is likely due to the short nature of most of the trials and because a number of the studies determined a hedonically acceptable maximum dose prior to the intervention (Yoshioka et al., 2004, Westerterp-Plantenga et al., 2005, Ahuja et al., 2007, Ludy and Mattes, 2011a). Indeed participants in one trial reported that ingestion of higher dosages over a long period would not be possible due to capsaicinoids' overpowering flavour and increases in gastric motility (dosage for this trial was 33mg/day in a predominantly Caucasian population) (Ahuja et al., 2007). Compliance issues were reported in a 12-week trial, featuring a much higher capsaicinoid dosage (135mg per day) and a number of participants were put on half dosages to allow them to continue in the trial (Lejeune et al., 2003).

It may be that rather than decreasing the actual amount of food eaten, capsaicinoid intake affected energy intake by changing food choice. Several studies observed a preference for carbohydrate-rich rather than fat-rich foods (Yoshioka et al., 1999, Yoshioka et al., 2004, Westerterp-Plantenga et al., 2005), which lead to the decrease in energy intake, rather than a reduction in the amount of food eaten. Such a change in food choice may be made possible by capsaicinoids influencing regions of the brain crucial to this process, notably the orbitofrontal cortex (Rolls, 2004b) and the hypothalamus (Baboota et al., 2014).

Several other mechanisms of action have been proposed for how capsaicinoids may cause this observed reduction in energy intake. This includes by stimulating the

release of a satiety inducing hormone (glucagon-like peptide-1) and reducing levels of a hunger promoting hormone (ghrelin) (Smeets and Westerterp-Plantenga, 2009). By the stimulation of the sympathetic nervous system, which may lead to a decrease in appetite (Wellman, 2000). Finally, capsaicinoids' may cause an increase in lipid oxidation, which is an important signaller for the control of energy intake (Leonhardt and Langhans, 2004); see section 2.7.2.3 for a detailed description of these effects.

Whether a reduction in energy intake would be maintained long term is not clear from the studies in this analysis, as most of the trials involved comparison of only two meals (one following capsaicinoid ingestion and one control meal). The trial with the longest intervention period, lasting 4 weeks, found no difference in energy intake between control and intervention groups (Ahuja et al., 2007). Two studies took into account whether participants were regular consumers of spicy foods prior to the trial, however results were contrasting. One trial finding a significant reduction in energy intake among regular consumers (Westerterp-Plantenga et al., 2005), the other finding a significantly reduction in energy intake only in non-regular consumers (Ludy and Mattes, 2011a). Also, what is considered regular consumption may vary greatly around the world, with average daily chilli consumption levels much higher among Asian populations (2.5-8g) than American and European populations (0.05-0.5g) (Gonlachanvit, 2010).

3.5 Summary

In summary, the analysis suggests there is a possibility capsaicinoids could play a role in weight management alongside exercise and other dietary measures. However, caution should be applied to this result however, due to the small size of the reduction and the short term nature of the trials involved. There was also likely to have been some publication bias in the final analysis, meaning that the size of capsaicinoids' effect on *ad libitum* energy intake may have been under-estimated therefore further trial publications should be encouraged. This study, taken together with the published literature suggest a dosage of above 2mg of capsaicinoids is needed to produce an effect and that a reduction in energy intake may be the result of a preference for carbohydrate rich foods over fat rich foods.

The extent of the effect on energy intake is relatively small and long term intake is likely to be required to produce a beneficial effect. It may also be the case that several doses a day are needed to produce a clinically meaningful effect on a person's weight; this may mean that capsaicinoids would need to be taken in the form of a supplement, as their strong sensory effect means most people would be unwilling to eat chillies with every meal. But combined with capsaicinoids' effect on energy intake and lipid oxidation observed in other research (Ludy et al., 2012, Whiting et al., 2012) there is potential for development of a natural weight loss aid, particularly as there currently are a lack of pharmaceutical treatment options for obesity.

Chapter 4 – Capsaicinoids and Body Fat in Caucasian Women: A Randomised Placebo Controlled Trial

4.1 Introduction

Previous research has suggested capsaicinoids intake may be associated with body composition (Whiting et al., 2012). Intervention trials have shown the acute effects of capsaicinoids intakes on increasing energy expenditure (Janssens et al., 2013) increasing fat oxidation (Ludy and Mattes, 2011b) and decreasing energy intake . However there is a paucity of data on longer-term effects and resulting body composition. The aim of the present study was to investigate the effects of capsaicinoid intake for six weeks with change in body fat as the primary outcome. Secondary outcomes were change in body weight, BMI, waist circumference, waist-to-hip ratio, BAI, blood glucose and blood cholesterol.

4.2 Study Design

We carried out a randomised placebo controlled parallel intervention trial of a capsaicinoid supplement for six weeks; with an allocation ratio of one-to-one. A parallel study design was used to prevent any potential carry over effect, due to change in body fat, biasing the outcome of the observations.

The study was conducted at Manchester Metropolitan University initially between February and May 2014 and then between August 2015 and February 2016. Participants for the trial were recruited in three waves via the Manchester Metropolitan University email system. After responding to the initial email, participants were then asked to complete a screening questionnaire, to assess their eligibility for inclusion in the trial (see section 4.4.1 for full details of the recruitment process). Those who met the eligibility criteria and consented to take part were invited to the initial measuring session. At baseline, 6 anthropometric measurements were taken along with a blood sample (see section 4.6 for details of measurements), before participants were randomised into the active intervention or placebo control groups. Participants were then given a supply of supplements, and asked to take two daily for six weeks. They

were also asked to complete a pill diary to assess compliance and a three-day, self-reported food diary during each week of the trial.

Further assessment of participants' anthropometric measurements and blood markers occurred at week three and week six, at which point supplementation finished. After a further three weeks without supplementation, a final follow-up assessment of anthropometric measurements and blood markers occurred (see figure 4.1). Statistical analysis was then undertaken to assess the statistical significance of any observed changes in the measurements, as a result of the intervention (see section 4.7.2).

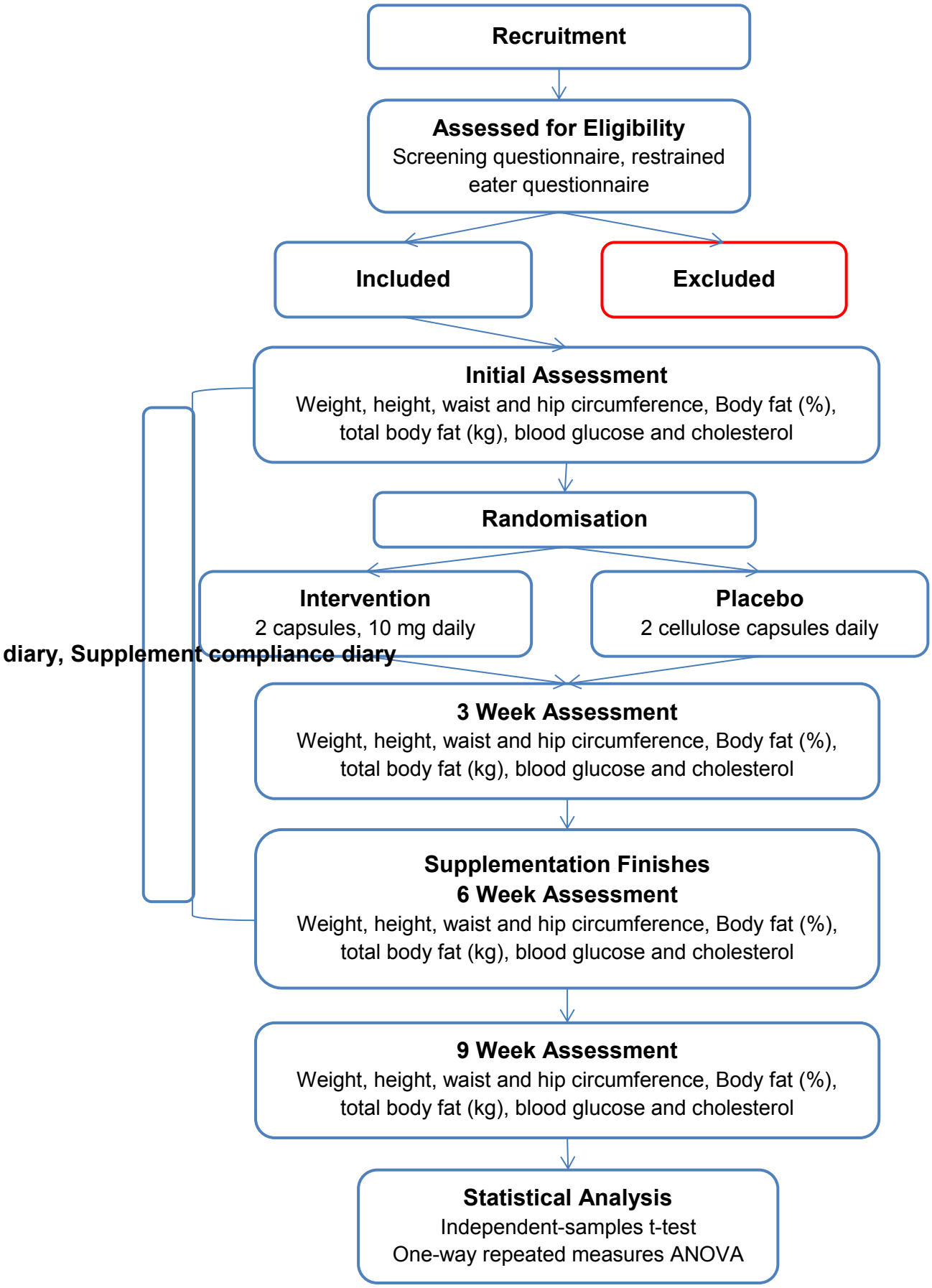


Figure 4.1 A flow chart outlining process of the trial from recruitment to completion.

4.2.1 Ethical Approval and Data Protection

Ethical approval was sought and granted from the Manchester Metropolitan University (Hollings Faculty) ethics committee (granted on 9th October 2013). Initially approval was for 30 participants, further approval to increase this number to 60 participants was granted in July 2015. The trial was conducted in accordance with the guidelines laid down the in the Declaration of Helsinki (World Medical Association, 2013). All participant data were kept confidential and in compliance with the Data Protection Act 1998. Paper records were kept in a locked, fire-proof cabinet and all computer records were stored on a password protected laptop.

4.2.2 Study Population

4.2.2.1 Recruitment

Initial interest in the trial was elicited via the email system of Manchester Metropolitan University. An advert was placed in a university newsletter (sent via email) to students and staff at the university (see appendix 1 for a copy of the email). Participants, who expressed an interest in joining the trial, were screened using a questionnaire to ensure they met the inclusion-exclusion criteria (see appendix 1 for a copy of screening questionnaire).

4.2.2.2 Inclusion-Exclusion Criteria

The inclusion-exclusion criteria were developed, based on the criteria used in similar capsaicinoid and weight loss trials (Lejeune et al., 2003, Ahuja et al., 2007, Snitker et al., 2009, Yang et al., 2012).

The study included women only to reduce any potential confounding effects due to gender differences in fat storage and metabolism. Evidence suggests there are pronounced differences in fat oxidation, circulating cholesterol levels, post-prandial fat oxidation and fat storage between men and women (Geer and Shen, 2009).

Participants aged 18-49 years old were selected, as persons under the age of 18 are often still in their growing phase (Eveleth and Tanner, 1976) and as such there is a possibility they could gain weight during the trial as a result of natural growth. Furthermore aging has been associated with changes in energy intake (Roberts, 2000)

and a decrease in energy expenditure (Roberts and Rosenberg, 2006) compared to earlier in a person's life.

Potential participants who were post-menopausal were excluded as research suggests there may be changes in the way the body accumulates adipose tissue after the menopause as opposed to before (Yannakoulia et al., 2007).

Potential participants who's BMI was less than 20kg/m^2 were excluded as any fat loss in this group could results in the participant becoming underweight and therefore placing them at risk of a complication.

Pregnant women were excluded due to the biological and physiological changes that occur during pregnancy.

Potential participants who were using other supplements and/or medication were excluded. An interaction between chillies intake and ACE inhibitor drugs has previously been reported (Fugh-Berman, 2000).

Potential participants who suffered from metabolic or digestive disorders were excluded. Capsaicinoids can have a strong effect on the digestive system, which may impact a person's disorder and worsen their symptoms.

Restrained eaters were excluded from the study as research suggests that restrained eaters are prone to over-eating as a result of this restraint (Lowe & Levine, 2005), and such over-eating may invalid the observed results of this trial. To assess whether a person was a restrained or non-restrained eater a validated questionnaire was used (Stunkard and Messick, 1985) (see appendix 1 for a copy of questionnaire).

4.2.3 Intervention

The intervention was given in the form of a supplement so the investigation could be conducted as a double-blind randomised control trial.

To determine the minimum dosage at which a biological effect is likely to occur, the results from a number of human trials that investigated the effect of capsaicinoids at different dosages were evaluated. In trials looking at potential weight loss effects, dosages have varied from a single 0.4mg dose (Reinbach et al., 2010) to 135mg/day

for 12 weeks (Lejeune et al., 2003). No effect was observed for the 0.4mg dosage; however trials giving a dosage of around 2 – 3mg/day have observed an effect (Westerterp-Plantenga et al., 2005, Yoshioka et al., 2004, Ludy and Mattes, 2011a, Whiting et al., 2014).

Compliance problems were reported in the highest dosage level trial (135mg/day with capsules) although what is acceptable may be influenced by participants' previous exposure and potentially their genetics. Exposure levels in countries with a high intake of chillies (such as Mexico, Japan, Korea) have reported intakes 25 - 200mg/person/day (Scientific Committee on Food, 2001), although intakes tend to be much lower among Western Caucasian population (Ludy and Mattes, 2011a). Therefore to provide a dosage that is likely to produce a measurable effect on body fat, without causing digestive side effects among the study population, a dosage of 10mg/day was used for the intervention (this was administered as 2 supplements containing 5mg of capsaicinoids).

Capsules were white (to conceal contents) and size 0. Participants were instructed to take 2 capsules daily, at separate times and to take them with meals (as capsaicinoids are fat soluble molecules). Participants completed a pill diary to monitor compliance.

4.2.3.1 Placebo

For this trial cellulose capsules were used for the placebo control and the size and colour of the capsules were identical to the intervention (size 0, white colour).

4.2.4 Outcomes

The a priori primary outcome was body fat (% and kg) and secondary outcomes were weight (kg), BMI (kg/m2), waist circumference (cm), hip-to-waist ratio, BAI, blood glucose (mmol/l) and blood cholesterol (mmol/l). All measurements were taken in the morning (between 8.30am and 10.30am) in a fasted state, following an overnight fast.

4.2.4.1 Anthropometric Measurements

Change in participants' body fat percentage and total body fat were calculated using bio-electrical impedance. Participants were asked not to eat, drink or perform strenuous physical activity in the 12 hours prior to arriving for measurements.

Participants' weight was recorded using a Tanita digital body composition scale (Tokyo, Japan) in kg to two decimal places. Participants were weighed barefoot, in light clothing, on a non-carpeted floor, 200g was subtracted from participants' weight to account for clothing.

Participant's height was measured barefoot on a non-carpeted floor using a Seca stadiometer (Hamburg, Germany), BMI was calculated as kg/m2.

Participants' waist and hip circumference measurements were taken according to the World Health Organisation guidelines (WHO, 2008). The position for measuring waist circumference was midway between the uppermost border of the iliac crest and the lower border of the costal margin (rib cage). The tape was placed around the abdomen at the level of this midway point and a reading was taken when the tape was snug but did not compress the skin. The participant stood with feet close together, arms at the side and wore minimal clothing. Measurements were taken at the end of a normal expiration. Each measurement repeated twice. If the measurements were within 1 cm of one another, the average was calculated. If the difference between the two measurements exceeded 1 cm, the two measurements were repeated. Using the same guidelines, hip circumference measurements were taken around the widest part of the buttocks. Waist-to-hip ratio was calculated as average waist (cm)/average hip (cm).

Body Adiposity Index is a recently developed measure of adiposity (Bergman et al., 2011) and is a way of estimating % body fat from height and hip circumference measurements; BAI was calculated using the following equation:

$$BAI = \left(^{\text{Hip circumference}} / {}_{\text{Height}}^{1.5}\right) - 18$$

4.2.4.2 Blood Measurements

All blood samples were taken by a trained by a trained phlebotomist, using a finger prick blood test, and blood glucose (measured in mmol/litre) and cholesterol levels (measured in mmol/litre) were analysed; samples were measured using an Analox GM7 analyser and disposed of immediately after analysis of the sample; all needles were disposed of in sharps bins.

4.2.5 Food Diaries

The participants were asked to fill out food diaries to ensure there was no difference in dietary intake between both intervention and placebo control groups. Participants were asked to keep diaries for three days (two weekdays and one weekend day of their choosing) of week 1, week 3 and week 6 of the trial. Participants were asked not to change their diets whilst being part of the trial. Food diaries were converted into nutrient intakes using WISP nutritional analysis software (version 3.0).

4.2.6 Sample Size

To perform an *a priori* sample size calculation, an estimate for the expected effect size was obtained using data from a previous study which measured change in body fat (kg) over multiple time points due to an intervention using a similar bioactive ingredient (green tea catechins) (Yang et al., 2012) (variance of the group means used was 2.35kg body fat).. An estimate of expected standard deviation was also required, however the aforementioned study recruited only participants with a BMI ≥25, so is unlikely to be representative of the standard deviations expected in this study. Therefore, an estimate of the expected standard deviation was taken from a previous intervention study (Ahuja et al., 2007) with similar inclusion-exclusion criteria to the present one (standard deviation used was (6.05kg). The calculation was performed using G-Power software (Faul et al., 2009), with the significance set to 5% and the power set to 90%. The a priori sample size estimation, for a one-way repeated measures ANOVA, was for 29 participants in the treatment group.

4.2.7 Randomisation

Stratified randomisation was used based on age and baseline BMI. Participants were placed into one of the following strata: Age <30 and BMI <26, Age ≥30 and BMI <26, Age ≥30 and BMI ≥26, Age <30 and BMI ≥26. A randomisation sequence was created for each group using the WinPepi program for epidemiologists (Abramson, 2011). In line with recruitment, randomisation was carried out in 3 waves.

4.2.8 Blinding Procedure

To ensure the intervention was double blind, randomisation and participant allocation to either placebo control or intervention group was carried out by the lead researcher.

A technician then placed supplements in unlabelled containers and marked either “A” or “B”, depending if they were placebo control or capsaicinoid capsules and the lead researcher handed out the supplements to the participants, according to the allocation.

4.2.9 Statistics

Means and standard deviations were calculated for all anthropometric measurements (body fat (% and kg), weight (kg), height (cm), BMI (kg/m2), waist circumference (cm), waist to hip ratio and body adipose index) and blood glucose (mmol/l) and blood cholesterol (mmol/l). Data on potential confounders (physical activity, income, alcohol intake, smoking and caffeine intake) was collected from participant questionnaires to allow comparisons between groups.

4.2.9.1 Interaction Analysis

The data for anthropometric and blood measurements was assessed for outliers by a visual inspection of the Q-Q plot and the distribution of the data was assessed using the Shapiro-Wilk test for normality. If there were no outliers and the data was normally distributed, a two-way ANOVA was performed to determine if there was any statistically significant interaction between the intervention and placebo control groups over time. With a p-value ≤0.05 considered to be statistically significant. If outliers were observed or the data was found to be not normally distributed, a non-parametric Friedman test was performed as an alternative. Mauchly's test of sphericity was used to test the assumption of sphericity, if the assumption was not met, the Greenhouse-Geisser method was used to correct the result (Greenhouse & Geisser, 1959).

4.2.9.2 Sensitivity Analysis

Again, the data for anthropometric and blood measurements was assessed for outliers by a visual inspection of the Q-Q plot and the distribution of the data was assessed using the Shapiro-Wilk test for normality. If there were no outliers and the data was normally distributed, a one-way repeated measures ANOVA was performed to determine if there was any statistically significant difference between the means over time. With a P value ≤0.05 considered to be statistically significant. If outliers were observed, a non-parametric Friedman test was performed as an alternative. If there were no outliers but the data was found to be not normally distributed, the repeated

measures ANOVA was still carried out, as the test is fairly robust to deviations from normality (Lix et al., 1996), Mauchly's test of sphericity was used to test the assumption of sphericity, if the assumption was not met, the Greenhouse-Geisser method was used to correct the result (Greenhouse and Geisser, 1959). Post-hoc dependent-samples t-tests were calculated for statistically significant results to identify at which measurement point changes occurred, with Bonferroni adjustment used to account for multiple t-tests.

Differences in the % change of mean values were also compared to baseline figures for each anthropometric and metabolic outcome and for each measurement (i.e. baseline v week 3, baseline v week 6 and baseline v week 9). An independent-samples t-test was used to compare the means. Outliers were assessed by a visual inspection of the data's Q-Q plots, the distribution of the data was assessed using the Shapiro-Wilk test for normality and the homogeneity of variances was assessed using Levene's test of equality of variances. If outliers were observed, a non-parametric Mann-Whitney U test was run as an alternative. If there were no outliers but the data was found to be not normally distributed, the independent samples t-test was still carried out, as the test is fairly robust to deviations from normality (Myers et al., 2010). If the assumption of homogeneity of variances was violated, a modified Welch t test was used to calculate p-values. A Bonferroni adjustment was used to correct for multiple t-tests.

To assess differences in dietary intake, nutritional data was compared over time using a one-way repeated measures ANOVA (using the same assumptions and procedures as stated above) and the intervention and placebo control groups were compared using an independent-samples t-test (using the same assumptions and procedures as stated above).

4.2.9.3 Intention-to-Treat Analysis

An intention-to-treat analysis, including all participants who were randomised and entered into the trial process was performed for all changes in anthropometric and metabolic measurements over time and percent change using the same methodology as described above. For participants who dropped out their final measurements (prior to withdrawal) were taken forward and used for the remainder of the trial.

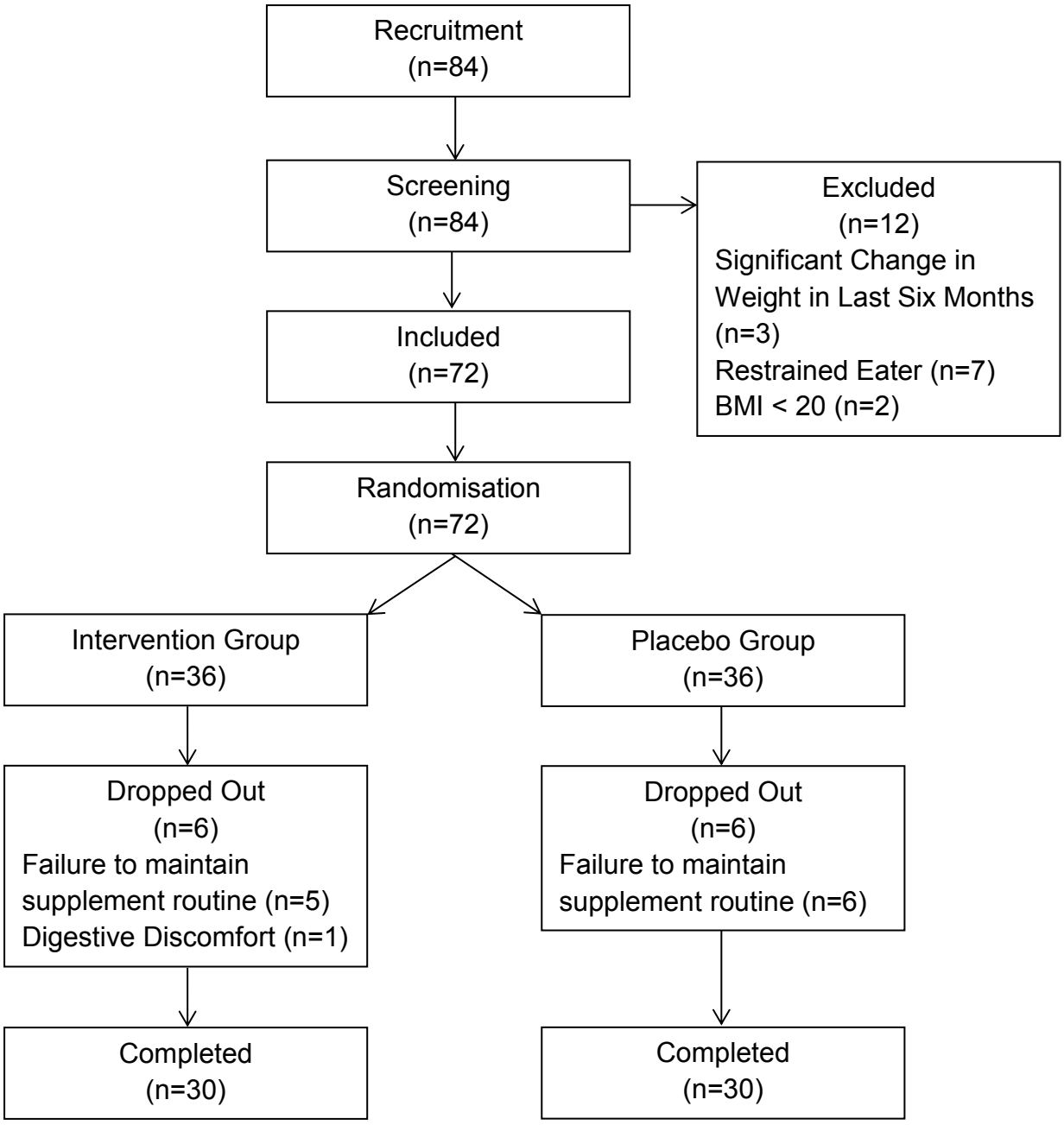
All analyses performed using SPSS statistical software (version 22.0) (IBM Corp., 2013).

4.3 Results

4.3.1 Recruitment and Randomisation

Following the email advertisement, 95 women expressed an interest in taking part in the trial and requested further information. Of these 11 women decided they would not be able to commit to the trial due to time commitments or for personal reasons. The remaining 84 entered the screening process. Details of exclusions, drop-outs and completers are shown in figure 4.2 below.

Figure 4.2 Recruitment, screening, drop-out and completion of participants.



4.3.2 Drop-out rate, compliance and tolerability

There was an 83.3% completion rate. Eleven participants dropped-out of the trial after failing to maintain the supplement taking regime or for personal reasons, the other drop out was a result reported of side effects. This participant reported strong stomach cramps after taking the supplements and therefore decided to withdraw from the trial. After revealing of blinding, it was shown she was taking the active capsaicinoid supplement.

All completing participants attended all four measuring sessions (baseline, week 3, week 6 and week 9) and completed and returned food diaries as requested. Supplement taking compliance was monitored via pill diary and all participants completed and returned this, reporting a compliance rate of 97% or above.

4.3.3 Baseline Characteristics

Participants' baseline age, anthropometric and metabolic measurements are presented in table 4.1 below. The randomisation process resulted in groups with largely similar age, anthropometric and metabolic measurements.

Table 4.1 Baseline age, anthropometric and metabolic measurements for all participants, placebo control group and intervention group.

Measure	All Participants		Placebo Control		Intervention	
	N	Mean (±Std. Deviation)	N	Mean (±Std. Deviation)	N	Mean (±Std. Deviation)
Age (yrs)	60	30 (±10)	30	29 (±10)	30	31 (±10)
Height (m)	60	1.65 (±0.07)	30	1.65 (±0.06)	30	1.66 (±0.07)
Body Fat (%)	60	32.5 (±7.5)	30	31.0 (±6.9)	30	34.1 (±7.9)
Body Fat (kg)	60	24.7 (±10.8)	30	22.6 (±10.4)	30	26.8 (±11.0)
Weight (kg)	60	72.95 (±15.57)	30	70.20 (±14.79)	30	75.69 (±16.08)
BMI (kg/m^2)	60	26.5 (±4.7)	30	25.5 (±4.5)	30	27.5 (±4.7)
Waist (cm)	60	84 (±11)	30	81 (±10)	30	86 (±12)
Ratio*	60	0.78 (±0.05)	30	0.77 (±0.04)	30	0.80 (±0.06)
BAI**	60	31.95 (±4.58)	30	31.35 (±4.34)	30	32.56 (±4.80)
Glucose (mmol/l)	60	4.4 (±0.8)	30	4.4 (±0.8)	30	4.5 (±0.7)
Cholesterol (mmol/l)	60	3.48 (±0.78)	30	3.40 (±0.76)	30	3.57 (±0.79)

*Ratio: waist circumference/hip circumference

**BAI: (hip circumference/height$^{1.5}$)-18

Probable confounders for the trial are presented in table 4.2 below. Results suggest the randomisation process resulted largely similar placebo control and intervention groups for the confounders assessed.

Table 4.2 A priori probable confounders at baseline for all participants, placebo control group and intervention group.

Confounder	Level	All Participants (N)	Placebo Control (N)	Intervention (N)
Physical activity at work	Sedate	16	7	9
	Moderate	29	16	13
	Active	15	7	8
Physical activity outside work	Sedate	22	10	12
	Moderate	28	15	13
	Active	10	5	5
Income (£000s)	0 - 15	27	14	13
	15 - 25	23	12	11
	25 - 50	9	4	5
	50+	1	0	1
Alcohol intake (units/wk)	0 - 10	34	18	16
	11 - 20	20	10	10
	21 - 30	5	2	3
	30+	1	0	1
Smoking	Smoker	13	6	7
	Non-smoker	47	24	23
Caffeine intake (caffeinated drinks/day)	0 - 3	30	16	14
	4 - 6	24	11	13
	7+	6	3	3

4.3.4 Dietary Intake

The analysis of participants' nutrient intake per day for the intervention and placebo control groups during the trial period are presented in table 4.3 below. There were no significant differences in mean nutrient intakes per day during the trial in either intervention or placebo control groups.

Table 4.3 Nutrient intake (per day) across the trial period for the intervention and placebo control groups along with p-values.

Group	Outcome	Week 1		Week 3		Week 6		p-value*
		Mean	SD	Mean	SD	Mean	SD	
Placebo Control	Energy (mJ)	8.21	0.67	7.97	0.41	8.08	0.63	0.266
	Protein (g)	86.1	11.9	83.5	9.4	84.7	11.9	0.426
	Fat (g)	68.7	10.6	66.8	9.9	67.6	9.9	0.292
	Saturated Fat (g)	24.1	4.4	23.4	4.0	23.7	4.4	0.280
	Monounsaturated fat (g)	22.8	3.7	22.1	3.0	22.4	3.5	0.502
	Polyunsaturated fat (g)	10.5	1.4	10.2	1.1	10.3	1.4	0.242
	Carbohydrates (g)	221.3	19.9	215.0	15.9	217.9	21.0	0.683
	Sugars (g)	88.7	13.9	86.1	12.3	87.4	15.1	0.875
	NEMS (g)	42.2	10.6	40.9	9.2	41.5	9.6	0.312#
	Starch (g)	123.5	13.8	120.0	12.1	121.5	13.4	0.189
	Alcohol (g)	9.5	7.8	9.8	8.3	9.7	8.8	0.701#
Intervention	Energy (kcal)	1928	116	1941	132	1918	122	0.581
	Energy (mJ)	8.10	0.48	8.15	0.55	8.05	0.51	0.582
	Protein (g)	84.3	10.2	85.0	11.5	84.0	10.8	0.555
	Fat (g)	68.6	13.1	68.9	12.6	68.0	11.8	0.516
	Saturated Fat (g)	27.2	8.9	27.4	8.9	27.0	8.7	0.415
	Monounsaturated fat (g)	24.1	5.2	24.2	5.2	23.9	5.0	0.430
	Polyunsaturated fat (g)	10.3	0.9	10.3	0.9	10.2	0.9	0.572
	Carbohydrates (g)	216.5	14.4	217.8	14.5	215.2	12.7	0.755
	Sugars (g)	86.3	9.3	86.8	9.3	85.7	8.6	0.356
	NMES (g)	42.7	7.5	43.0	7.9	42.5	7.8	0.234#
	Starch (g)	123.1	7.9	123.9	8.1	122.4	7.2	0.851
	Alcohol (g)	9.2	8.5	9.6	8.9	10.0	8.1	0.798#

*p-values obtained using a one-way repeated measures ANOVA unless indicated otherwise
#p-values obtained using a non-parametric Friedman test (used due to outlying data)

A comparison of the mean dietary intakes of the intervention and placebo control groups during the trial period is presented in table 4.4 below, with comparative p-values. Results show there were no significant differences in mean nutrient intakes per day during the trial between the intervention and placebo control groups.

Table 4.4 Comparison of mean nutrient intakes per day during the trial period in the intervention and the placebo groups.

Outcome	Placebo		Intervention		p-value*
	Mean	SD	Mean	SD	
Energy (MJ)	8.08	0.34	8.10	0.42	.805
Protein (g)	84.5	9.8	84.4	10.4	.971
Fat (g)	67.7	9.3	68.5	12.3	.781
Saturated Fat (g)	23.8	4.0	27.2	8.8	.055
Monounsaturated fat (g)	22.4	3.2	24.1	5.1	.137
Polyunsaturated fat (g)	10.4	1.2	10.3	0.8	.750
Carbohydrates (g)	218	14	217	11	.641
Sugars (g)	87.4	12.9	86.3	8.5	.696
NMES (g)	41.6	9.5	42.7	7.6	.595
Starch (g)	121.7	11.1	123.1	6.3	.544
Alcohol (g)	9.7	8.8	9.6	9.0	.984

*p-values obtained using an independent-samples t-test

4.3.5 Effect of the Intervention

4.3.5.1 Interaction Analysis

Results of the anthropometric and metabolic measurements for all outcomes during the nine-week trial period are presented in table 4.5 (below). P-values suggest there was no significant interaction between the groups over time for any outcome measured.

Table 4.5 Anthropometric and metabolic measurements at each assessment point during the 9 week trial period for intervention and placebo control groups.

Outcome	Placebo Control				Intervention				p-value# group	p-value# time*group
	Baseline Mean (± Std. Deviation)	Week 3 Mean (± Std. Deviation)	Week 6 Mean (± Std. Deviation)	Week 9 Mean (± Std. Deviation)	Baseline Mean (± Std. Deviation)	Week 3 Mean (± Std. Deviation)	Week 6 Mean (± Std. Deviation)	Week 9 Mean (± Std. Deviation)		
Body Fat (%)	31.0 (±6.9)	31.3 (±7.1)	31.1 (±7.2)	31.0 (±7.1)	34.1 (±7.9)	34.2 (±7.5)	33.4 (±7.5)	33.6 (±7.6)	0.004	0.996
Body Fat (kg)	22.6 (±10.4)	22.8 (±10.6)	22.8 (±10.8)	22.7 (±10.9)	26.8 (±11.0)	26.8 (±10.6)	26.1 (±10.5)	26.3 (±10.6)	0.007	0.998
Weight (kg)	70.20 (±14.79)	70.21 (±14.79)	70.55 (±15.03)	70.55 (±15.30)	75.69 (±16.08)	75.49 (±16.17)	75.29 (±16.04)	75.38 (±16.02)	0.012	0.999
BMI (kg/m^2)	25.5 (±4.5)	25.5 (±4.5)	25.7 (±4.6)	25.7 (±4.7)	27.5 (±4.7)	27.4 (±4.7)	27.3 (±4.7)	27.4 (±4.7)	0.003	0.998
Waist (cm)	81 (±10)	81 (±11)	82 (±10)	82 (±10)	86 (±12)	87 (±13)	86 (±13)	87 (±14)	0.001	0.983
W-H Ratio*	0.77 (±0.04)	0.77 (±0.04)	0.78 (±0.04)	0.77 (±0.04)	0.80 (±0.06)	0.80 (±0.06)	0.79 (±0.06)	0.80 (±0.08)	0.001	0.890
BAI**	31.35 (±4.34)	31.37 (±4.45)	31.43 (±4.50)	31.58 (±5.35)	32.56 (±4.80)	32.67 (±4.50)	32.60 (±4.60)	32.68 (±4.47)	0.047	1.000
Glucose (mmol/l)	4.4 (±0.8)	4.3 (±0.6)	4.4 (±0.7)	4.3 (±0.7)	4.5 (±0.7)	4.4 (±0.6)	4.6 (±0.7)	4.4 (±0.7)	0.277	0.939
Cholesterol (mmol/l)	3.40 (±0.76)	3.36 (±0.78)	3.18 (±0.61)	3.26 (±0.57)	3.57 (±0.79)	3.49 (±0.80)	3.14 (±0.43)	3.30 (±0.59)	0.675	0.925

*Ratio: waist circumference/hip circumference

**BAI: (hip circumference/height1.5)-18

#p-vaules obtained using a two-way ANOVA

4.3.5.2 Sensitivity Analysis

Results of the anthropometric and metabolic measurements for all outcomes during the nine-week trial period are presented in table 4.6 (below). P-values suggest there was significant differences in the intervention group over time for the primary outcome of body fat (% and kg) and blood cholesterol. There were no significant differences over time for other anthropometric and metabolic measurements.

Table 4.6 Anthropometric and metabolic measurements at each assessment point during the 9 week trial period for intervention and placebo control groups.

Group	Outcome	Baseline Mean (± SD)	Week 3 Mean (± SD)	Week 6 Mean (± SD)	Week 9 Mean (± SD)	p-value[#]
Placebo Control	Body Fat (%)	31.0 (±6.9)	31.3 (±7.1)	31.1 (±7.2)	31.0 (±7.1)	0.468
	Body Fat (kg)	22.6 (±10.4)	22.8 (±10.6)	22.8 (±10.8)	22.7 (±10.9)	0.628
	Weight (kg)	70.20 (±14.79)	70.21 (±14.79)	70.55 (±15.03)	70.55 (±15.30)	0.730
	BMI (kg/m^2)	25.5 (±4.5)	25.5 (±4.5)	25.7 (±4.6)	25.7 (±4.7)	0.800
	Waist (cm)	81 (±10)	81 (±11)	82 (±10)	82 (±10)	0.536
	W-H Ratio*	0.77 (±0.04)	0.77 (±0.04)	0.78 (±0.04)	0.77 (±0.04)	0.710
	BAI**	31.35 (±4.34)	31.37 (±4.45)	31.43 (±4.50)	31.58 (±5.35)	0.817
	Glucose (mmol/l)	4.4 (±0.8)	4.3 (±0.6)	4.4 (±0.7)	4.3 (±0.7)	0.480
	Cholesterol (mmol/l)	3.40 (±0.76)	3.36 (±0.78)	3.18 (±0.61)	3.26 (±0.57)	0.114
Intervention	Body Fat (%)	34.1^{a} (±7.9)	34.2^{ab} (±7.5)	33.4^{ac} (±7.5)	33.6^{ac} (±7.6)	0.001
	Body Fat (kg)	26.8^{a} (±11.0)	26.8^{a} (±10.6)	26.1^{b} (±10.5)	26.3^{ab} (±10.6)	0.001
	Weight (kg)	75.69 (±16.08)	75.49 (±16.17)	75.29 (±16.04)	75.38 (±16.02)	0.260
	BMI (kg/m^2)	27.5 (±4.7)	27.4 (±4.7)	27.3 (±4.7)	27.4 (±4.7)	0.169
	Waist (cm)	86 (±12)	87 (±13)	86 (±13)	87 (±14)	0.445
	W-H Ratio*	0.80 (±0.06)	0.80 (±0.06)	0.79 (±0.06)	0.80 (±0.08)	0.530
	BAI**	32.56 (±4.80)	32.67 (±4.50)	32.60 (±4.60)	32.68 (±4.47)	0.904
	Glucose (mmol/l)	4.5 (±0.7)	4.4 (±0.6)	4.6 (±0.7)	4.4 (±0.7)	0.074
	Cholesterol (mmol/l)	3.57^{a} (±0.79)	3.49^{a} (±0.80)	3.14^{b} (±0.43)	3.30^{ab} (±0.59)	0.005

*Ratio: waist circumference/hip circumference

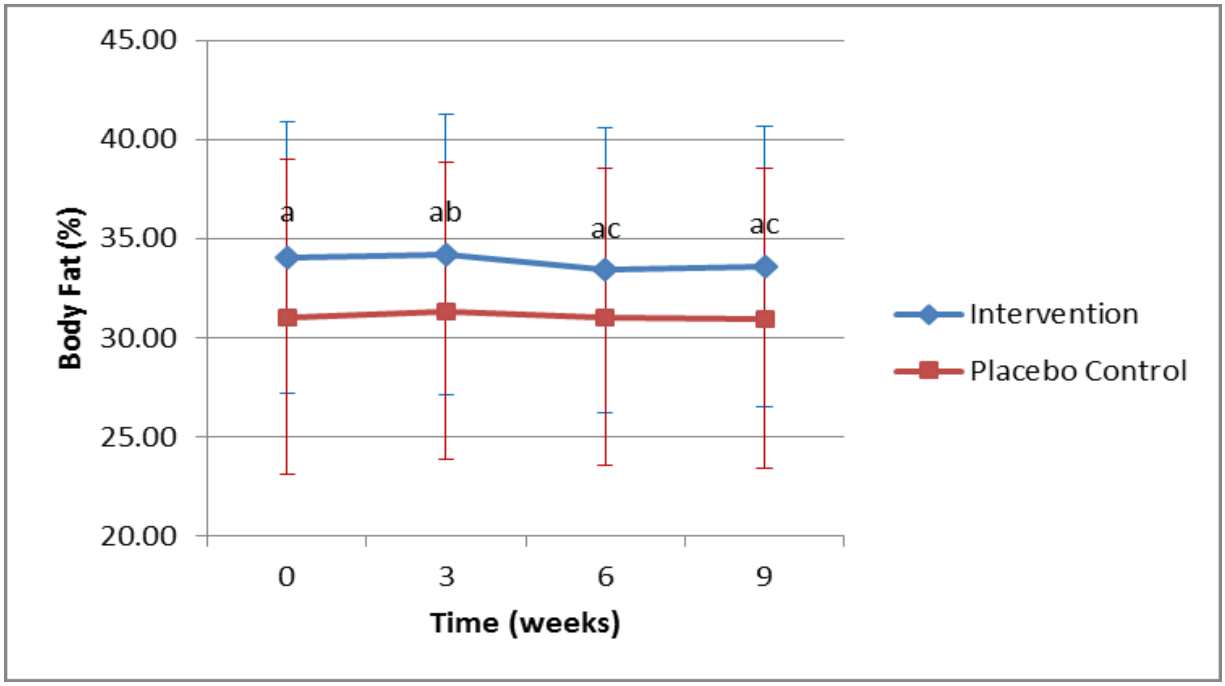
**BAI: (hip circumference/height1.5)-18

[#]p-values obtained using a one-way repeated measures ANOVA

^{a,b,c} depict statistical difference between measurements obtained using a dependent-samples t-test (Bonferroni adjusted)

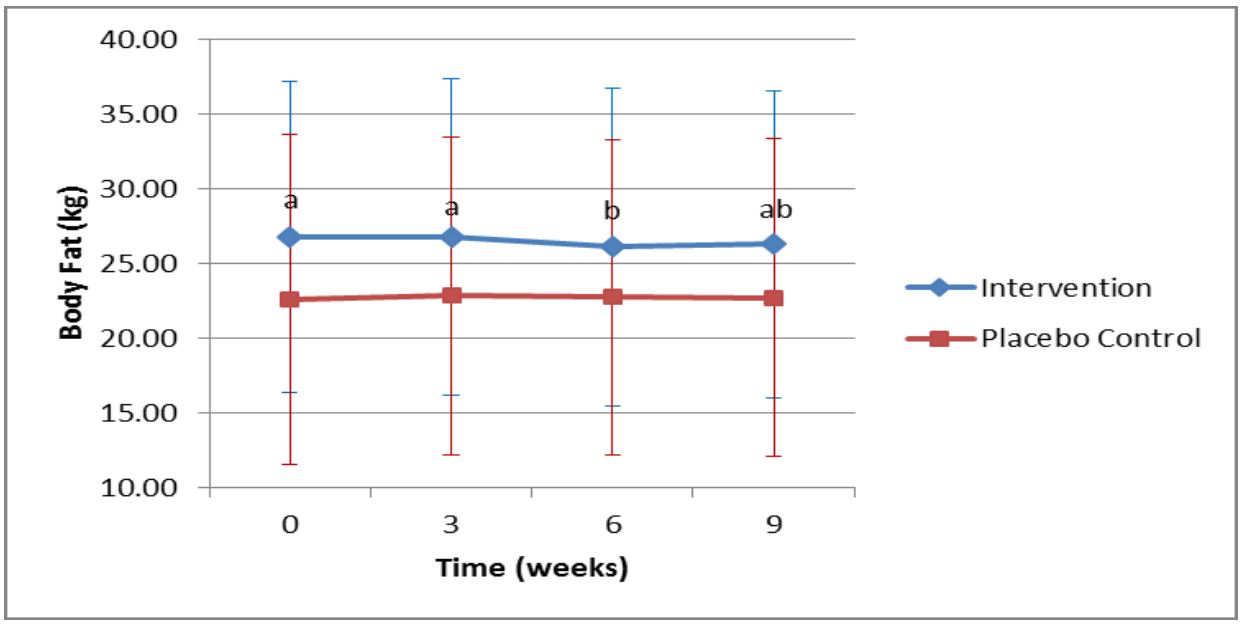
Graphs for statistically significant findings are presented below.

Figure 4.3 Change in body fat (%) during the trial period in the intervention and placebo control groups (n=60) with error bars indicating standard deviation.



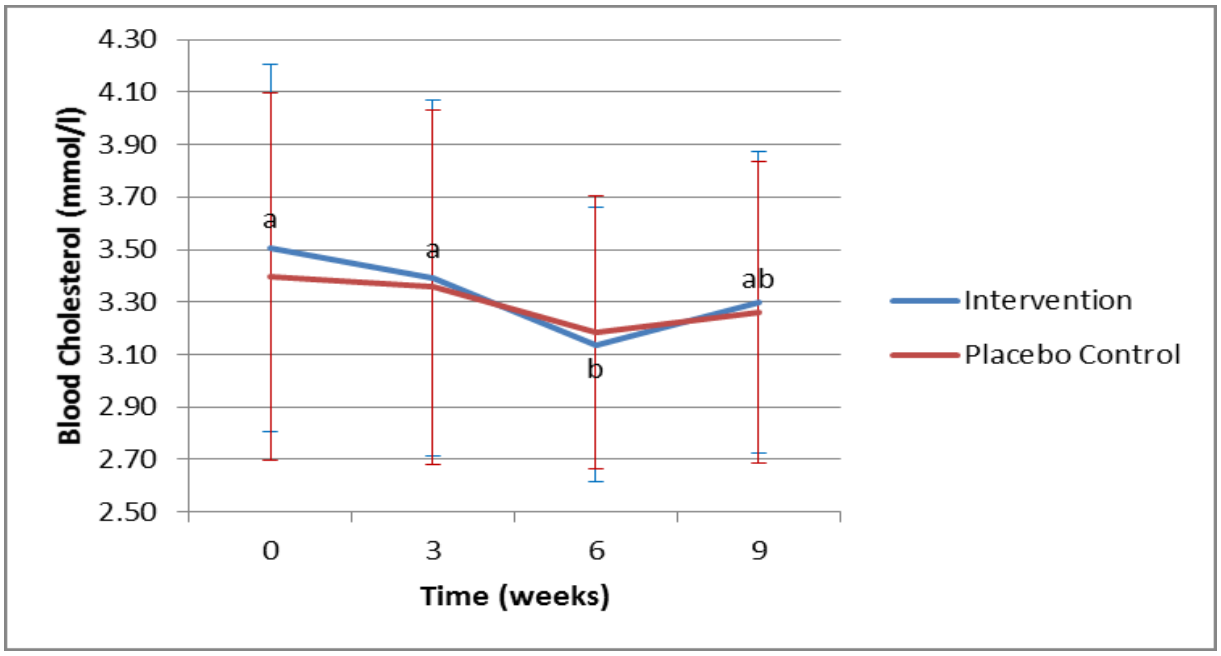
[a,b,c] depict statistical difference between measurements obtained using a dependent-samples t-test (Bonferroni adjusted)

Figure 4.4 Change in body fat (kg) during the trial period in the intervention and placebo control groups (n=60) with error bars indicating standard deviation.



[a,b,c] depict statistical difference between measurements obtained using a dependent-samples t-test (Bonferroni adjusted)

Figure 4.5 Change in blood cholesterol (mmol/l) during the trial period in the intervention and placebo control groups (n=60) with error bars indicating standard deviation.



[a,b,c] depict statistical difference between measurements obtained using a dependent-samples t-test (Bonferroni adjusted)

Calculations for % change for all outcomes at week 3, 6 and 9 compared to baseline are presented in table 4.7 (below) for both intervention and placebo control groups. Results indicate there are no significant difference in percentage change across outcomes during the trial period.

Table 4.7 Percent change at weeks 3, 6 and 9 compared to baseline for all anthropometric and metabolic outcomes for placebo control and intervention groups, along with comparative p-values.

Comparison v baseline	Group						p-value*#
	Placebo Control			Intervention			
	N	Mean	SD	N	Mean	SD	
% Fat percentage change week 3	30	1.04	5.03	30	0.85	4.41	0.847
% Fat percentage change week 6	30	0.01	5.02	30	-1.52	4.48	0.218
% Fat percentage change week 9	30	-0.09	6.91	30	-1.02	3.62	0.517
% Fat (kg) change week 3	30	1.06	5.22	30	0.59	4.94	0.720
% Fat (kg) change week 6	30	0.48	5.23	30	-2.07	4.71	0.053
% Fat (kg) change week 9	30	0.38	7.84	30	-1.51	4.12	0.247
% Weight change week 3	30	0.01	1.18	30	-0.29	1.18	0.322
% Weight change week 6	30	0.47	1.39	30	-0.56	1.41	0.006
% Weight change week 9	30	0.42	1.75	30	-0.43	1.83	0.071
% BMI change week 3	30	0.01	1.18	30	-0.42	1.47	0.213
% BMI change week 6	30	0.47	1.39	30	-0.68	1.78	0.007
% BMI change week 9	30	0.42	1.75	30	-0.49	2.29	0.089
% Waist circumference change week 3	30	0.57	4.93	30	0.25	3.81	0.780
% Waist circumference change week 6	30	1.22	4.75	30	-0.70	5.36	0.146
% Waist circumference change week 9	30	1.02	5.18	30	0.42	7.32	0.713
% Waist-Hip ratio change week 3	30	0.50	4.49	30	-0.05	3.46	0.601
% Waist-Hip ratio change week 6	30	1.09	4.93	30	-0.87	4.21	0.105
% Waist-Hip ratio change week 9	30	0.71	5.47	30	0.03	5.61	0.637
% BAI change week 3	30	0.19	4.93	30	0.57	4.37	0.756
% BAI change week 6	30	0.37	5.42	30	0.34	5.68	0.987
% BAI change week 9	30	0.66	6.83	30	0.60	4.99	0.967
% Blood glucose change week 3	30	-0.95	11.35	30	-1.25	13.54	0.926
% Blood glucose change week 6	30	3.04	16.19	30	4.93	17.45	0.665
% Blood glucose change week 9	30	0.23	13.55	30	-1.71	14.83	0.600
% Blood cholesterol change week 3	30	-0.70	10.58	30	-1.77	10.28	0.693
% Blood cholesterol change week 6	30	-4.27	15.65	30	-9.53	16.68	0.213
% Blood cholesterol change week 9	30	-1.82	15.81	30	-5.28	17.80	0.429

#p-values obtained using an independent-samples t-test
*Statistical significance was set at p≤ 0.002 to account for multiple testing (27 tests)

4.3.5.3 Intention-to-Treat Analysis

The baseline age, anthropometric and metabolic measurements for the intention-to-treat analysis are presented in table 4.8 below for all participants, placebo control and intervention groups. The randomisation process resulted in groups with largely similar age, anthropometric and metabolic measurements.

Table 4.8 Intention-to-treat analysis for anthropometric and metabolic measurements at each assessment point during the 9 week trial period for intervention and placebo control groups.

Measure	All Participants		Placebo Control		Intervention	
	N	Mean (±SD)	N	Mean (±SD)	N	Mean (±SD)
Age (yrs)	72	30 (±10)	36	29 (±10)	36	30 (±10)
Height (m)	72	1.66 (±0.06)	36	1.65 (±0.06)	36	1.66 (±0.07)
Body Fat (%)	72	32.5 (±7.1)	36	31.6 (±6.6)	36	33.4 (±7.5)
Body Fat (kg)	72	24.5 (±10.1)	36	23.02 (±9.8)	36	26.0 (±10.3)
Weight (kg)	72	72.97 (±14.54)	36	70.78 (±13.94)	36	75.17 (±14.97)
BMI (kg/m^2)	72	26.4 (±4.4)	36	25.8 (±4.2)	36	27.1 (±4.7)
Waist (cm)	72	83 (±11)	36	81 (±10)	36	85 (±11)
Ratio*	72	0.78 (±0.05)	36	0.77 (±0.04)	36	0.79 (±0.06)
BAI**	72	31.61 (±4.47)	36	31.23 (±4.25)	36	31.98 (±4.71)
Glucose (mmol/l)	72	4.4 (±0.7)	36	4.4 (±0.8)	36	4.5 (±0.7)
Cholesterol (mmol/l)	72	3.44 (±0.69)	36	3.43 (±0.78)	36	3.44 (±0.65)

*Ratio: waist circumference/hip circumference

**BAI: (hip circumference/$height^{1.5}$)-18

A comparison of baseline age, anthropometric and metabolic measurements for all participants who were randomised and all participants who completed the trial is presented in table 4.9 below. P-values suggested there was no statistically significant difference between all randomised and all completing participants for age, anthropometric and metabolic measurements.

Table 4.9 Intention-to-treat analysis comparing baseline age, anthropometric and metabolic measurements for all randomised participants and all completing participants.

Measure	All Randomised		All Completers		p-value[#]
	N	Mean (±Std. Deviation)	N	Mean (±Std. Deviation)	
Age (yrs)	72	30 (±10)	60	30 (±10)	0.872
Height (m)	72	1.66 (±0.06)	60	1.65 (±0.07)	0.744
Body Fat (%)	72	32.5 (±7.1)	60	32.5 (±7.5)	0.991
Body Fat (kg)	72	24.5 (±10.1)	60	24.7 (±10.8)	0.909
Weight (kg)	72	72.97 (±14.54)	60	72.95 (±15.57)	0.891
BMI (kg/m^2)	72	26.4 (±4.4)	60	26.5 (±4.7)	0.795
Waist (cm)	72	83 (±11)	60	84 (±11)	0.663
Ratio*	72	0.78 (±0.05)	60	0.78 (±0.05)	0.970
BAI**	72	31.61 (±4.47)	60	31.95 (±4.58)	0.922
Glucose (mmol/l)	72	4.4 (±0.7)	60	4.4 (±0.8)	0.927
Cholesterol (mmol/l)	72	3.44 (±0.69)	60	3.48 (±0.78)	0.919

[#]p-values calculated using an independent-samples t-test
*Ratio: waist circumference/hip circumference
**BAI: (hip circumference/$height^{1.5}$)-18

Results of the anthropometric and metabolic measurements for all outcomes, for all randomised participants during the trial period are presented in table 4.10 (below). P-values suggest there was significant differences in the intervention group over time for the primary outcome of body fat (% and kg) and blood cholesterol. There were no significant differences over time for other anthropometric and metabolic measurements.

Table 4.10 Intention-to-treat analysis with anthropometric and metabolic measurements for all outcomes during the 9 week trial period for placebo control and intervention groups (n=36 for all measurements).

Group	Outcome	Baseline Mean (± SD)	Week 3 Mean (± SD)	Week 6 Mean (± SD)	Week 9 Mean (± SD)	p-value[#]
Placebo Control	Body Fat (%)	31.6 (±6.6)	31.8 (±6.8)	31.6 (±6.9)	31.5 (±6.8)	0.462
	Body Fat (kg)	23.02 (±9.8)	23.2 (±10.0)	23.2 (±10.2)	23.1 (±10.3)	0.638
	Weight (kg)	70.78 (±13.94)	70.79 (±13.95)	71.07 (±14.14)	71.07 (±14.38)	0.074
	BMI (kg/m^2)	25.8 (±4.2)	25.8 (±4.3)	25.9 (±4.3)	25.9 (±4.4)	0.081
	Waist (cm)	81 (±10)	82 (±10)	82 (±10)	82 (±10)	0.507
	W-H Ratio*	0.77 (±0.04)	0.78 (±0.05)	0.78 (±0.04)	0.78 (±0.05)	0.667
	BAI**	31.23 (±4.25)	31.25 (±4.34)	31.30 (±4.38)	31.42 (±5.12)	0.817
	Glucose (mmol/l)	4.4 (±0.8)	4.3 (±0.6)	4.5 (±0.7)	4.4 (±0.7)	0.480
	Cholesterol (mmol/l)	3.43 (±0.78)	3.40 (±0.76)	3.25 (±0.63)	3.32 (±0.59)	0.115
Intervention	Body Fat (%)	33.4^{a} (±7.5)	33.5^{ab} (±7.1)	32.9^{ac} (±7.5)	33.0^{ac} (±7.1)	0.003
	Body Fat (kg)	26.0^{a} (±10.3)	26.0^{a} (±10.0)	25.4^{b} (±9.9)	26.3^{ab} (±10.6)	0.004
	Weight (kg)	75.17 (±14.97)	75.01 (±15.04)	74.84 (±14.93)	74.91 (±14.90)	0.206
	BMI (kg/m^2)	27.1 (±4.7)	27.0 (±4.5)	27.0 (±4.4)	26.4 (±5.8)	0.300
	Waist (cm)	85 (±11)	86 (±12)	85 (±12)	86 (±13)	0.444
	W-H Ratio*	0.79 (±0.06)	0.79 (±0.06)	0.79 (±0.06)	0.79 (±0.07)	0.530
	BAI**	31.98 (±4.71)	32.07 (±4.50)	32.01 (±4.55)	32.08 (±4.44)	0.904
	Glucose (mmol/l)	4.5 (±0.7)	4.4 (±0.7)	4.6 (±0.7)	4.4 (±0.7)	0.075
	Cholesterol (mmol/l)	3.44^{a} (±0.65)	3.35^{a} (±0.58)	3.14^{b} (±0.46)	3.27^{ab} (±0.59)	0.007

*Ratio: waist circumference/hip circumference

**BAI: (hip circumference/height1.5)-18

[#]p-values obtained using a one-way repeated measures ANOVA

[a,b,c] depict statistical difference between measurements (Bonferroni adjusted)

Calculations for % change for all outcomes at week 3, 6 and 9 compared to baseline are presented in table 4.11 (below) for all randomised participants, in both intervention and placebo control groups, along with comparative p-values. Results indicate there are no significant difference in percentage change across outcomes during the trial period.

Table 4.11 Intention-to-treat analysis for % change at weeks 3, 6 and 9 compared to baseline for all anthropometric and metabolic outcomes for placebo control and intervention groups, along with comparative p-values.

Comparison v baseline	Group						p-value*#
	Placebo Control			Intervention			
	N	Mean	SD	N	Mean	SD	
% Fat percentage change week 3	36	0.87	4.60	36	0.71	4.02	0.847
% Fat percentage change week 6	36	-0.06	4.59	36	-1.27	4.12	0.242
% Fat percentage change week 9	36	-0.07	6.29	36	-0.85	3.32	0.510
% Fat (kg) change week 3	36	0.89	4.77	36	0.49	4.52	0.720
% Fat (kg) change week 6	36	0.34	4.80	36	-1.72	4.36	0.061
% Fat (kg) change week 9	36	0.33	7.13	36	-1.26	3.80	0.244
% Weight change week 3	36	0.01	1.07	36	-0.24	1.07	0.321
% Weight change week 6	36	0.39	1.28	36	-0.46	1.30	0.007
% Weight change week 9	36	0.34	1.60	36	-0.36	1.67	0.071
% BMI change week 3	36	0.01	1.07	36	-0.35	1.35	0.212
% BMI change week 6	36	0.39	1.28	36	-0.57	1.64	0.007
% BMI change week 9	36	0.35	1.60	36	-0.49	1.98	0.192
% Waist circumference change week 3	36	0.47	4.49	36	0.21	3.46	0.779
% Waist circumference change week 6	36	1.02	4.32	36	-0.59	4.88	0.145
% Waist circumference change week 9	36	0.85	4.72	36	0.35	6.66	0.712
% Waist-Hip ratio change week 3	36	0.42	4.09	36	-0.04	3.15	0.600
% Waist-Hip ratio change week 6	36	0.90	4.50	36	-0.72	3.85	0.104
% Waist-Hip ratio change week 9	36	0.59	4.98	36	0.03	5.10	0.636
% BAI change week 3	36	0.16	4.49	36	0.47	3.98	0.755
% BAI change week 6	36	0.31	4.93	36	0.29	5.17	0.987
% BAI change week 9	36	0.55	6.22	36	0.50	4.54	0.967
% Blood glucose change week 3	36	-0.80	10.33	36	-1.04	12.33	0.926
% Blood glucose change week 6	36	2.53	14.78	36	4.10	15.99	0.666
% Blood glucose change week 9	36	0.19	12.33	36	-1.42	13.51	0.599
% Blood cholesterol change week 3	36	-0.58	9.63	36	-1.98	9.78	0.543
% Blood cholesterol change week 6	36	-3.55	14.34	36	-7.11	14.69	0.302
% Blood cholesterol change week 9	36	-1.51	14.40	36	-3.49	15.62	0.579

#p-values obtained using an independent-samples t-test

*Statistical significance was set at p≤ 0.002 to account for multiple testing (27 tests)

Chapter 5 – Discussion

5.1 Intervention Trial

To date, there have been 18 published intervention trials investigating capsaicinoids' effect on weight management outcomes. Despite many trials observing positive effects, there is a paucity of data on longer-term effects and resulting body composition. The present intervention trial was intended to assess the potential impact that capsaicinoids could have on body fat following a six week capsaicinoid intervention. Of the published trials, only two of those trials featured multi-week interventions. One of which was a weight regain trial (Lejeune et al., 2003) and therefore difficult to assess capsaicinoids effect on body fat due to rapid weight loss. The other trial's primary outcome was the effect on metabolic and arterial function and featured a crossover design less suited to observing changes in body fat. The present trial was therefore the first to assess whether a multi-week capsaicinoid intervention could reduce body fat. However, interaction analysis found no significance change between the placebo control and intervention groups over time for any of the outcomes measured.

5.1.1 Primary Outcome

The interaction analysis found no significance change between the placebo control and intervention groups over time for body fat percentage ($p = 0.996$) or total body fat ($p = 0.998$), suggesting the intervention had no effect on these outcomes. There was a significant between the groups for both body fat percentage ($p = 0.004$) and total body fat ($p = 0.007$). This significant difference in adiposity levels between the two groups suggests that the randomisation created uneven groups with regard to body fat levels.

However, the sensitivity analysis found that after six weeks of supplementation, the capsaicinoid group had a small but statistically significant reduction in mean body fat percentage of 0.64% ($p = 0.022$) and a small but statistically significant decrease in mean total body fat of 0.67kg ($p = 0.007$). There were no significant changes in body fat percentage or total body fat in the placebo control group, who remained relatively stable throughout the trial; suggesting than the observed change in body composition

maybe due to the capsaicinoid intervention. The contrasting results between the interaction analysis and the sensitivity analysis however, suggest this finding lacks robustness. These contrasting results could be due to a lack of statistical power (that the number of participants taking part was too small to observe a significant effect) or because there was a difference in adiposity levels between the two groups, which was larger than the change in adiposity observed over time.

A number of previous trials, including both short-term and long-term interventions, have found capsaicinoids to increase lipid oxidation (Lejeune et al., 2003, Janssens et al., 2013), this is also the case with capsinoid trials (Lee et al., 2010b, Josse et al., 2010). However, what has not been seen from these trials was whether this increase in lipid oxidation would lead to a beneficial change in body composition (i.e. less adipose tissue). Previous non-capsaicinoid research has found an association between lipid oxidation and the amount of adipose tissue in humans (Schutz et al., 1992) and it has also been observed in a number of animal studies that capsaicinoid ingestion could beneficially alter body composition (Sambaiah and Satyanarayana, 1982), however, a significant effect has not been observed in humans, prior to the present investigation.

A previous multi-week study monitored change in body fat as a result of a capsaicinoid intervention but found no difference in body fat percentage and total body fat between the capsaicin and placebo groups (Lejeune et al., 2003). However, that was a weight regain study, and had a significantly different methodology which would likely impact any observed changes in body fat. Participants lost around 7-8% of body weight (in a four-week very low calorie diet) before starting supplementation, and both groups' body fat percentage and total body fat were lower at the end of the trial than they were at the start, despite gaining weight during the supplementation period. In addition to this, the trial estimated fat free mass and body fat percentage using equations with estimates derived from total body water, a technique which has been shown to result in less accurate calculations of body fat percentage (Sen et al., 2010). This would be a potential problem for short-to-medium length trials as changes in body fat due to capsaicinoids are likely to be relatively small. Therefore, it is difficult to assess whether the results of the aforementioned trial are directly comparable to this results of the present one.

Another multi-week capsaicinoid trial (four-week intervention) assessed change in body fat and observed no significant effect due to the intervention (Ahuja et al., 2007). However, that trial had a cross-over design, with no wash out period whose primary outcome was to investigate capsaicinoids' effect on metabolic and arterial function. A cross-over design is unlikely to be conducive to detecting changes in body composition, which are slower to occur than metabolic and arterial outcomes and more likely to be affected by the impact of the participants' being under both control and intervention conditions.

Two human trials using capsinoids have investigated whether they could affect body composition. A short term trial (two weeks), found capsinoids (again ingested as food) found a decrease in body fat percentage (-0.31%), although this was not statistically significant ($p > 0.05$) (Kawabata et al., 2006). This lack of significance may have been due to the short intervention period. A longer-term capsinoid trial (intervention lasting 12-weeks), failed to find a benefit in terms of body fat percentage or body composition, with both placebo and intervention groups losing around 1% of body fat during the intervention period. However, the capsinoid group did lose significantly higher amounts of abdominal fat compared to the placebo group ($p = 0.049$). Abdominal adipose tissue was not recorded in the present study, which used bio-electrical impedance to measure adipose tissue, whereas the prior study used dual energy X-ray absorptiometry, which measures both total and site specific adiposity. It was not therefore possible to compare this aspect of the two trials' results.

Capsaicinoids may be influencing adipose tissue levels by several different mechanisms. Stimulation of TRPV1 receptors, leading to increased activity of the sympathetic nervous system, may cause the body to mobilise adipose stores for energy usage, leading to an increase in lipid oxidation and an overall reduction in adipose tissue (Bartness et al., 2010). Stimulation of TRPV1 channels by capsaicinoids may induce calcium influx into cells, which has been demonstrated to prevent adipogensis, potentially preventing adipose cells from forming (Zhang et al., 2007). Capsaicinoids may also be causing this effect by altering the expression of adipose cell protein and enzymes and altering adipokine secretion (Joo et al., 2010). Finally, capsaicinoids may stimulate and de-sensitive nerve cells leading to changes in adipose tissue formation and distribution (Stearns et al., 2012).

Also possible is that capsaicinoids may be aiding muscle hypertrophy, leading to lower body fat percentages and higher lean mass percentages, with one study finding that capsaicin treatment stimulated skeletal muscle hypertrophy in mice (Ito et al., 2013). Mechanical load-induced intracellular signalling events are important for subsequent skeletal muscle hypertrophy and researchers have found that in the mammalian body, muscle hypertrophy required a TRPV1-mediated increase in intracellular calcium concentrations. In addition, capsaicin treatment could stimulate the same pathway, mimicking the signalling events caused by mechanical loading and causing a significant increase in skeletal muscle.

5.1.2 Secondary Outcomes

5.1.2.1 Weight

The results suggest that over a six-week intervention period, capsaicinoid supplementation had no statistically significant effect on total body weight. Weight in the capsaicinoid group declined slightly after 6 weeks of supplementation (-0.40kg from baseline), however this change was not statistically significant, with neither the interaction analysis between the two groups over time ($p = 0.999$) or the sensitivity analysis ($p = 0.260$) finding a significant effect. Such a change in weight is small in the context of natural human weight fluctuations. Human body weight can vary due to a number of factors including hydration status, food intake, salt intake and menstrual cycle (Bunt et al., 1989). Human body weight is known to fluctuate by 2% or more on a daily basis (Vivanti et al., 2013), which for the participants in the trial would mean daily variations of around 1.5kg. Therefore, it would seem the capsaicinoid intervention had minimal effect on body weight following 6 weeks of supplementation.

It may be that a longer intervention period would be required to observe an effect on body weight. Two trials involving capsinoid interventions both found that medium-term supplementation (12-weeks) increased energy expenditure by around 50kcal/day (Snitker et al., 2009, Galgani and Ravussin, 2010). For this relatively small increase in energy expenditure and to aid weight loss would take time. Taking the mean weight (72.95kg) and age (29.95) of participants in this trial, a clinically significant weight loss (considered to be 5-10% (Douketis et al., 2005)) would be 3.5kg - 7.0kg. Using weight loss modelling (Hall and Jordan, 2008), this kind of weight loss would take at least 539

days (77 weeks). It may be the case therefore that the trial was not long enough to detect changes in body weight.

Capsaicinoids may also be able to aid reductions in body weight via limiting of energy intake. This effect has been observed in intervention trials (Janssens et al., 2014) and meta-analysis (Whiting et al., 2014); it may occur through action on intestinal satiety hormones (Smeets and Westerterp-Plantenga, 2009), stimulation of the sympathetic nervous system (Yoshioka et al., 1999), its effect on the brain, specifically the orbitofrontal cortex (Rolls, 2004a) or the hypothalamus (Baboota et al., 2014), or by increasing lipid oxidation (Leonhardt and Langhans, 2004). However, like energy expenditure, this effect is small; estimates from the meta-analysis suggested a decrease in energy expenditure of 251kJ (Whiting et al., 2014). Using weight loss modelling, a clinically significant weight loss (5-10%) would take at least 358 days (51 weeks). Again this is suggestive, that if capsaicinoid supplementation were to lead to a beneficial change in body weight, the present trial was too short to detect any effect.

It is also possible that changes in substrate oxidation due to the intervention may affect body weight measurements. Capsaicinoids have been observed to increase lipid oxidation and decrease carbohydrate oxidation in both short-term (Janssens et al., 2013, Shin and Moritani, 2007, Yoshioka et al., 1998) and long-term intervention trials (Lejeune et al., 2003). As the body utilises higher levels of adipose stores (i.e. increased lipid oxidation), this may result in an increase in body weight, particularly over the short-term (Maughan et al., 2007). Carbohydrates are stored in the body as glycogen and these stores typically included about 3 or 4 times the weight of glycogen in water (Pocock et al., 2013). It is assumed that when these glycogen stores are used, the associated water then becomes available to the body and therefore quickly lost; thus the body utilising 0.5kg of glycogen for energy usage, would result in a weight loss of around 2 – 2.5kg, while utilising of adipose stores would result in a lower reduction of body weight (Garrow and Summerbell, 1995). In addition to this, substrate utilisation results in high levels of carbon dioxide creation, with lipid oxidation creating nearly twice the amount of CO_2 as carbohydrate and protein oxidation. As a result lipid oxidation actually results in a temporary net gain in body mass as the mass of carbon dioxide generated is less than the mass of oxygen consumed (Maughan et al., 2007). The overall effect of this is that elevated levels of lipid oxidation, which capsaicinoid

supplementation may be causing, could result in slightly higher body weight. Although this effect would be small and temporary (particularly as CO_2 is expired), potentially though it may have had an impact on body weight levels observed in the trial.

5.1.2.2 BMI

There was no statistically significant change in BMI in either group throughout the trial. Neither the interaction analysis between the two groups over time ($p = 0.998$) or the sensitivity analysis ($p = 0.169$) finding a significant effect. BMI did decrease in the intervention group at week 6 by 0.18kg/m^2, compared to baseline ($p = 0.480$). However, the lack of statistical significance and the decrease being very small is suggestive that six weeks of capsaicinoid supplementation at this dosage had no effect on this measure. This is not surprising as any change in BMI would be related to change in body weight. Like for changes in body weight, it is not obvious from the trial whether the lack of change in BMI is a result of there being no effect from capsaicinoid supplementation or the effect being relatively small and the trial being too short to detect it. For an objective assessment of the effects of capsaicinoids on BMI a longer term trial would be required.

5.1.2.3 Waist circumference and waist-to-hip ratio

There were no significant changes in waist circumference in either group during the trial. Neither the interaction analysis between the two groups over time ($p = 0.983$) or the sensitivity analysis ($p = 0.445$) finding a significant effect. There was also no significant change in waist-to-hip circumference in either group, with neither interaction analysis between the two groups over time ($p = 0.890$) or the sensitivity analysis ($p = 0.530$) finding a significant effect. Mean waist and hip circumferences in the capsaicinoid group fluctuated above and below the baseline value during the trial by less than 1cm; with changes not being statistically significant ($p > 0.05$). In terms of the waist-to-hip ratio there were no significant changes in either group during the trial period. Suggesting the six week capsaicinoid intervention had no effect on these measures.

Waist circumference is considered to be an indicator of body fat, especially visceral adipose tissue (Rankinen et al., 1999). Therefore, it may be expected that a decrease

in percentage and total body fat (as observed in this trial) would be reflected in waist circumference measurements, however this was not the case. It may be concluded that adipose tissue lost was not around the visceral area, or that the waist circumference is not a sensitive enough measurement to detect the small decrease in body fat (observed via bio-electrical impedance measurements), following six weeks of supplementation.

Waist circumference is a marker prone to variation due to human measurement error and natural variation of the human body and therefore less reliable than other measurements used in this trial such as weight and body fat (Bigaard et al., 2005, Panoulas et al., 2008). In addition to this it is a measurement that is known to vary in humans seasonally by several centimetres (Visscher and Seidell, 2004), short term variations can also occur due to the respiratory cycle (by between 3 – 8cm) (Pellowe et al., 2010), changes due to the menstrual cycle (Kirchengast and Gartner, 2002) and due to the effects of food, faeces and flatus (Pellowe et al., 2010), therefore it may not be sensitive enough to detect the small changes in adiposity observed.

Another trial using capsinoid found a reduction in abdominal obesity following 12-weeks of supplementation (Snitker et al., 2009), so it may be expected that a reduction in waist circumference would be observed in this trial. That trial used Dual-energy X-ray absorptiometry scanning, which is a more sensitive instrument than waist circumference to changes in visceral adiposity, and also involved equal amounts of both male and female participants, unlike the present trial which involved only women. Waist measurement may be a poorer indicator of overall adiposity in women than in men (Camhi et al., 2011), with males more likely to store adipose tissue around the visceral area than females (Ayonrinde et al., 2011). Thus, any adipose tissue lost in the present population group is likely to be less than an all-male or mixed gender participant group. Therefore, waist circumference may not be the most reliable indicator of adipose tissue levels in the present population group, making it difficult to draw conclusions from the observed small changes in waist circumference.

5.1.2.4 Body Adiposity Index

There were no statistically significant variations in mean body adiposity index (BAI) from baseline value throughout the trial. Observed changes were relatively small and

combined with the lack statistical significance with neither the interaction analysis between the two groups over time (p = 1.000) or the sensitivity analysis (p = 0.530) finding a significant effect. Suggesting the capsaicinoid intervention had little effect on this measure of body adiposity. These values reflect the small changes in hip circumference observed during the trial, as this measure is based around a calculation which uses height and hip circumference. BAI is a relatively recently created measure, designed to give an estimation of body adiposity from basic anthropometric measurements (Bergman et al., 2011). In this trial mean BAI was consistently close to mean percentage body fat (as measured by bio-electrical impedance), being no more than 1.5% away at any point in the trial. On an individual basis however, BAI was more prone to variations; the highest difference was 8.01% between the two figures (33.89 for BAI vs 41.9% for body fat percentage). So although a relatively accurate guide on a population basis, these individual variations make picking up subtle changes in adiposity less reliable using this measure.

5.1.3 Biochemical analysis

5.1.3.1 Fasting Blood Cholesterol

The interaction analysis found no significance change between the placebo control and intervention groups over time for fasting blood cholesterol levels (p = 0.925), suggesting the intervention had no effect on this outcome. There were however statistically significant changes in the intervention group for mean fasting blood cholesterol levels observed in the interaction analysis. Mean levels decreased 0.37mmol/l (from baseline value) at week six (p = 0.006). A similar pattern was also observed in the placebo control group, although the differences were smaller and did not reach statistical significance (p = 0.114). The contrasting results between the interaction analysis and the sensitivity analysis however, suggest this finding lacks robustness. These contrasting results could be due to a lack of statistical power, the number of participants taking part being too small to observe a significant effect in the interaction analysis.

A number of research studies have investigated the potential of capsaicinoids to affect blood cholesterol levels in animal models, however results have been inconclusive (Zhang et al., 2013). Capsaicinoids favourably modified the lipoprotein profile in

hamsters over a six-week period and rats given capsaicinoid supplementation for 28 days (Zhang et al., 2013). Another study found a cholesterol lowering effect due to capsaicin supplementation in mice fed a high fat diet (Shubha et al., 2011). Conversely, another trial reported no effect on cholesterol following capsaicinoid supplementation (Srinivasan and Chandrasekhara, 1992). Little research has been done into the effect on cholesterol levels in humans, although one trial observed no effect following a four-week capsaicinoid supplemented diet (Ahuja et al., 2007).

The results observed in this trial raise the possibility that capsaicinoids may have a cholesterol lowering effect in humans, however further research is required to investigate this possibility further. It should be noted that participants in the present trial would not be a group considered at risk of cardiovascular related health problems due to their cholesterol levels, with all mean and individual participants levels within the healthy range of values (Truswell, 2010). Also blood cholesterol levels are prone to short-term variations due to a number of factors including genetics and diet, specifically the amount of animal fats consumed (Willett, 2013) and are prone to much larger variations than anthropometric measurements. For example, in the present trial around a 10% reduction was seen in mean blood cholesterol in this trial, whereas a 10% decrease in body weight would be considered a large variation.

5.1.3.2 Fasting Blood Glucose

There were no statistically significant changes in mean fasting blood glucose observed throughout the trial observed in either the interaction analysis between the two groups over time or the sensitivity analysis. Mean blood glucose fluctuated above and below the baseline level in both groups during the nine weeks of the trial and it appears than the capsaicinoid intervention had no significant effect on blood glucose during the trial. Values for mean blood glucose remained within the normal range for non-diabetics throughout the trial (4.0-5.9mmol/litre) (Ceriello et al., 2014), so it appears that six weeks of capsaicinoid supplementation had no adverse effects on this measure.

Research in animal models suggests capsaicinoids can have a significant influence on blood glucose and insulin levels. Dietary capsaicin not only improved glucose tolerance and increased insulin levels but also lowered daily blood glucose profiles in mice (Wang et al., 2012) and capsaicin has been demonstrated to increases glucose

uptake in muscle cells, also in mice (Kim et al., 2013). However, another capsaicinoid intervention trial in humans which measured changes in blood glucose, found similar results to the present study; with no statistically significant difference between control and intervention arms of the trial (Ahuja et al., 2007).

5.1.4 Effect on Appetite and Energy Intake

It has been observed in several previous research studies that capsaicinoids added to the diet my lead to a reduction in energy intake and thereby aiding weight loss (Janssens et al., 2014, Ludy and Mattes, 2011a). Although the present study was conducted to assess changes in body fat, energy intake was recorded via food questionnaires, therefore it is possible to examine if capsaicinoids had any effect. In terms of energy intake, both groups had very similar intake levels, which did not significantly change over time during the trial period. This contrasts to data from the meta-analysis on energy intake, which suggested that capsaicinoid ingestion could cause a reduction in energy intake of 251kJ (60kcal) (Whiting et al., 2014); however care needs to be taken when drawing conclusions from this data.

Food diaries are prone to under-reporting and data may be compromised due to recall problems or social desirability bias (i.e., misreporting on sensitive or embarrassing behaviours to appear more favourable) (Leatherdale and Laxer, 2013), making energy intake figures for the present trial prone to bias. The methodology used here was also different to other trials investigating this area, which have not relied upon questionnaires, but supplied participants with food and measured how much was consumed (Westerterp-Plantenga et al., 2005). It may well be the case that food diaries are simply not a sensitive enough instrument to detect the small changes in energy intake that might potentially have occurred as a result of capsaicinoid ingestion.

Using Henry energy equations (Henry, 2005) and mean values of age, weight and height of each group (at baseline) can give an estimate of the energy requirements (assuming seated work and light exercise). Doing so suggests the capsaicinoid group should be consuming 2138kcal/day to maintain their current weight and the placebo control group 2072kcal/day. Based on reported intakes, the capsaicinoid group would have had an energy deficit of 870kJ/day (208kcal/day) and the placebo control group a deficit of 586kJ/day (140kcal/day). With such a calorie deficit, particularly for the

capsaicinoid group, it would be expected to see a larger reduction in body weight during the trial, than was observed. This suggests a potential lack of reliability in the reporting of energy intake in the food diaries in this study. Therefore, it makes it difficult to draw any conclusion concerning capsaicinoids' effect on energy intake from the results of this investigation.

5.1.5 Intention-to-treat Analysis

An intention-to-treat analysis was carried out to assess whether any bias was introduced into the trial observations due to participant drop-outs. It would appear from analysis however that minimal bias was introduced due to drop-outs. The randomisation process resulted in groups with largely similar age, anthropometric and metabolic measurements. There were no statistically significant differences when comparing the mean baseline age, anthropometric and metabolic measurements of all randomised participants verses all completing participants. Together, this suggests that at baseline the intervention and placebo control groups were largely similar across all measurements and that participant drop out had no impact on this.

Re-analysing the effect of the intervention including the final measurements of those who dropped out had minimal impact on the findings. There was still a statistically significance difference over time for the measurements of body fat (%) and body fat (kg) and for cholesterol, with no differences observed for any other outcomes. The analysis also indicated there are no significant difference in percentage change across outcomes during the trial period. These results were similar to the analysis that didn't include participants that dropped-out. Therefore, it's unlikely that any bias was introduced into the results of the trial due to participants dropping out.

5.1.6 Placebo Control Group

It was expected that as fat loss is outside of conscious control, there would be no evidence of fat loss in the placebo control group. The placebo control group was therefore considered a reference group. Placebo effects that have been observed in previous research trials are primarily related to subjective and self-appraised symptoms (such as pain relief), rather than the causation of permanent physiological changes to the body that are measurable (Kaptchuk and Miller, 2015). Statistical analysis of the results of the trial was therefore carried out in accordance with this

assumption, by initially using a repeated measures one-way ANOVA to assess change in outcomes over time and then analysing the differences in the % change of mean values over time. Analysis of the placebo control group found no difference in any of the outcomes over time, suggesting the placebo supplement produced no measurable effect on the outcomes investigated.

5.2 Limitations of the Research

Though informative the present trial has a number of limitations. Results of the interaction analysis suggest there were significant differences between the placebo control and intervention group among all anthropometric measurements ($p < 0.05$). Therefore, comparison of changes between the two groups over time as a result of the intervention are difficult to make. For example, for the primary outcome, the difference between the two groups in terms of mean body fat percentage at baseline was 3.1%. Body fat percentage changed by no more than 0.7% in either group drawing the trial, a much smaller amount than the difference between the two groups. This may explain the differences observed by the interaction analysis which found no significant change over time and the sensitivity analysis which did find a significant change in the intervention group. It appears the randomisation procedure failed to achieve balanced placebo control and intervention groups for anthropometric measures. Participants were randomised according to age and BMI. It may have been more appropriate to randomise the groups via the primary outcome measure of body adiposity.

For an investigation concerning the effects of changes in body fat the trial was relatively short. Changes in body composition take a long time and a particular problem for weight loss research is weight regain tends to occur in the years following the completion of the intervention (Strohacker et al., 2013). There is some evidence the weight loss benefits of capsaicinoids become lessened over time with regular consumption (Ludy and Mattes, 2011a), although this effect has not been observed in all studies, notably a number investigations in Asian populations who were already regular consumer prior to the trials (Yoshioka et al., 1995, Yoshioka et al., 1998, Yoshioka et al., 2004). The overall result would have been more informative and robust, if it had occurred over a longer period of time. Particularly advantageous would information about the effect on body weight, for which a longer trial would be required.

This was however, a pilot study with limitations on time and finance and the intervention was long enough to observe a statistically significant change in body fat.

The study utilised a parallel design as opposed to a crossover, where participants undergo both placebo control and intervention conditions. Parallel studies have the advantage of having a higher statistical power, due to more measurements being taken with the same number of participants, therefore are more likely to make accurate observations. However, the main drawback is the requirement for a washout period where the effects of the intervention leave the body before participants begin the next condition. Although a relatively short washout period would be required before capsaicinoids would be undetectable in the blood, their potential effect on body composition may last much longer. In was determine therefore that due to potential changes in body composition and their long lasting effect on the human body, a parallel design where participants only undergo one condition would be the most suitable design to test the hypothesis.

The research was carried out in participants who had a BMI of 20 and above, therefore it involved both normal weight and overweight participants (BMI ranged from 20.12 – 41.41kg/m^2). As an investigation into capsaicinoids' potential effects for aiding loss of fat, the principal population group that capsaicinoids would be aimed at would be overweight persons. Therefore, the result of the trial may would have been more informative about capsaicinoids' effect in this population group had the research population only contained overweight participants. Those of a normal weight were included in the research to aid recruitment and ensure an adequate number of participants were recruited to meet the *a priori* statistical power calculation.

The research was only carried out in female participants, therefore it is not clear if similar results would be observed in males. Other research trials in this area have used female only (Yoshioka et al., 1999), male only (Yoshioka et al., 2004) and mixed gender population groups (Janssens et al., 2014) in their investigations and there has been no apparent pattern of gender difference. It is likely therefore that the results found in the present study would be applicable to both males and females. An all-female population group was chosen to limit potential confounders in the observations

made as a result of the intervention, but leaves open the possibility that the effect may be different in differing genders.

With an all-female population group, effects of the menstrual cycle on the observed measurements need to be considered. Prior research has found that the menstrual cycle can have an impact on energy intake and potentially the weight of participants during intervention trials (Buffenstein et al., 1995). Typical menstrual cycles are round 28 days (Singh et al., 2008), therefore during the six-week intervention period, participants may have been through different stages of their menstrual cycles and this may have had an impact on food intake, potentially this may have introduced some bias into the trial. The robustness of the observations may have been improved having an eight-week intervention, ensuring the majority of participants passed through two complete cycles and minimising any potential impact on the results.

The research was only carried out in Caucasian participants, therefore it is not obvious from this trial whether the result could be repeated in other population groups. Habitual dietary intake of capsaicinoids varies greatly around the world, with a large difference between Caucasian and Asian populations (López-Carrillo et al., 2003). Whether habitual intake may have an effect on capsaicinoids' action is unclear, although there is some evidence of genetic adaptations among Asian populations to green tea (Hursel and Westerterp-Plantenga, 2010), another food commonly consumed in Asia, but less so amongst Caucasians, therefore it is a possibility. This population group was chosen to limit potential confounders in the observations made as a result of the intervention, but leaves open the possibility that the effect may be different in differing ethnic groups.

There was a reliance on self-reporting for some aspects of the study; notably historical recall, especially changes in weight, reporting of restrained eating tendencies (using the three factor eating questionnaire) and during the trial, in particularly food and supplement diaries. Although not ideal, historical screening for changes in weight and eating habits, using questionnaires, does provide a way to assess these measures in a simple and time efficient manner; it does however leave open the possibility of recruitment of participants who may not be suitable.

Participants were not included in the trial if their weight had fluctuated more than 3kg in the past six months. However, as it was self-reported it assumes that participants

are regularly monitoring their weight prior to commencement of the trial, which may not be the case for everyone. Indeed, only three of the 84 participants who entered the screening process reported a change in weight of more than 3kg in the past six months. An improvement to the study design would have been to have a lead-in period in which to measure participants' weight several weeks before commencement of the intervention and excluding those with a large change at baseline; although this would make the trial longer and costlier to run. In terms of actual fluctuations during the trial, as a group the participant population did remain relatively weight stable throughout the trial; with the mean of both placebo control and intervention groups not fluctuating by more than 0.4kg, suggesting the study population was relatively weight stable.

Adherence of supplement taking was also self-reported, using supplement diaries, which were chosen as a simple and cost effective way of monitoring. It is possible to use more accurate measure such as pill bottles with digital lids, that record whenever they are opened (Dang, 2012), unfortunately these were beyond the budget for the trial. Another method to monitor compliance would be to monitor blood or urine levels of capsaicinoids. Research capsaicinoids have a short life in the blood stream, before being broken down (Weerapan, 2009) so it is not clear if this would be easy to detect. Research from animals has found capsaicinoids detectable in urine (Kawada et al., 1984), so this may potentially be an improved methodology.

Self-reported adherence of supplement taking was high at over 97%; this is much higher than has been reported in a review of compliance rates for prescription medicine involving 76 healthcare studies, which observed rates of 51% - 79% (Schmitz et al., 2005), with forgetfulness is generally sited as the major cause of failure to comply (Vervloet et al., 2012). A high compliance rate is crucial for any trial to successfully assess an intervention and a weakness of the present study is reliance on participant self-reporting.

Self-reporting was also used to monitor food intake during the trial; via 3-day food diaries, recorded weekly. The use of self-reported food diaries in other research trials have been shown to have a degree of unreliability, particularly under-reporting of energy intake (Ambrosini et al., 2009); accuracy may be compromised due to recall problems or social desirability bias (i.e., misreporting on sensitive or embarrassing

behaviours to appear more favourable) (Leatherdale and Laxer, 2013). Therefore, the accuracy of reporting of food intake in the present study is uncertain. Self-recorded food diaries were also not accurate enough to test the potential effects of capsaicinoids on energy intake. Although changes in energy intake have been observed in several trials due to capsaicinoid interventions, the changes are too small to be reliably observed via food diaries, so this could not be assessed during this intervention.

In order to accurately test the effects of the intervention, participants were asked to maintain their current diet and exercise regime during the trial. Assuming participants were weight stable prior to commencement of the intervention and that they did not change either their dietary or exercise habits, any changes observed changes in body composition could be attributed to the intervention. Unfortunately, there was no cost effective way for this variable to be controlled for during the present trial. Other research studies, similar to the present one, have tried to control for energy intake by supplying participants with food throughout the trial (Yang et al., 2012). While this would certainly have produced a more reliable way of controlling energy intake, it was unfortunately outside the scope of the finances for this investigation. A weakness of this research therefore, is reliance on participants' complying with the request to maintain their normal diet and exercise regime.

Although it is common for dietary trials to ask participants to maintain their normal diet during a research trial, it's possible that these instructions may have impacted the energy intake figures for the trial. Participants potentially may have followed their regular eating habits to comply with the instructions rather than following their hunger. Therefore, the potential energy intake reducing effect of capsaicinoids may have been negated. The instructions to maintain a normal diet were however chosen as the act of keeping a food diary can have a significant impact on dietary intake by itself (Neuhouser et al., 2008) and may have introduced bias into the results of the trial if the instruction to maintain normal dietary habits was not given.

The trial used bio-electrical impedance as the main measure of changes in participants' body composition during the trial. However, the accuracy of bio-electrical impedance has been questioned in some studies due to its reliance on population-specific equations to calculate body composition (Böhm and Heitmann, 2013); this is

thought to most likely to problematic in obese persons, whose body fat distribution may vary significantly from norms (Pimentel et al., 2010). Therefore, it could be argued that use of a more reliable method of measuring body composition would have produced a more accurate result in the present study; especially as observed changes in body composition were small. In addition, use of dual energy X-ray absorptiometry would also have provided information concerning distribution of body fat. Bio-electrical impedance was chosen as it is relatively quick and non-invasive compared to other ways of measuring (such as air displacement plethysmography or dual energy X-ray absorptiometry), which may have helped improve participant recruitment and retention. Facilities for the use of this method were also readily available at the university's physiology laboratory.

A methodological improvement that may have aided the accuracy of the measurements would have been to asked participants to urinate prior to measurements, to ensure a more consistent level of water in the body during the measurements. Hydration levels can affect bio-electrical impedance readings (Sun et al., 2005) and therefore may have impacted upon observations from the trial. Measurements were taken first thing in the morning after an overnight food and liquid fast, but asking participants to urinate prior to the measurements may have aided accuracy.

During the trial a number of different anthropometric measurements were recorded (in the case of weight, hip and waist circumference and body fat level) and calculated (in the case of BMI, BAI and hip/waist ratio). Although measurements of participants' body fat percentage and total body fat showed a statistically significant change, this was not reflected in the other anthropometric measurements. If this was the case it would have added more weight to the hypothesis that capsaicinoid supplementation can cause a beneficial change in body composition. It could be argued therefore that this is increases the probability that the observed change was due to chance, rather than the intervention. However, the observed change in body adiposity was small and the other the other anthropometric measures may not have been sensitive enough to detect this change. Waist and hip measurements are reliant on human measuring consistency and subject to relatively large natural fluctuations (Bosy-Westphal et al., 2010) and the calculated indicators, BAI and hip/waist ratio, are also reliant on these measures.

Overall the results of the trail suggest capsaicinoids interventions could have the potential to aid weight management outcomes. Results from this research study suggest that supplementation with capsaicinoids may produce a small decrease in body fat when ingested for six weeks. This decrease is probably too small to provide any immediate health benefit and supplementation would need to continue for longer periods and to be done in conjunction with diet and exercise interventions.

5.3 Thesis Discussion

5.3.1 Introduction

This research project was intended to assess the potential impact that capsaicinoids could have on weight management outcomes. Specifically, to perform a systematic literature search of human intervention trials investigating the effects on weight management outcomes and to collate and analyse findings into a review paper. Following this it was found there was conflicting data as to capsaicinoids effect on energy intake and a meta-analysis summarising the results of intervention trials in this area was undertaken. In addition, despite there being numerous short-term intervention trials with promising results, there had also been a lack of longer-term intervention trials investigating capsaicinoids potential effect on body composition. Therefore, a double-blind, placebo controlled intervention study to investigate the effects of capsaicinoids on body fat was undertaken. This section will review the main findings of the thesis, what conclusions can be drawn from this research and recommendations for future research will also be discussed. Findings will also be critically examined in light of previous research around this subject.

5.3.2 Current Obesity Treatments

There is currently a lack of viable options for the treatment of obesity. Intensive lifestyle interventions, consisting of dietary restriction and physical activity/exercise accompanied with behaviour therapy have proven to be largely ineffective (Mann et al., 2007). Even with longer term interventions (six months and over), once the intervention period finishes compliance with improved lifestyle is poor. The result is generally a transient phase of weight loss followed by a return on the trajectory towards obesity (Kushner, 2013). Anti-obesity drug development programmes have been littered with false starts, failures in clinical development, and withdrawals due to

adverse effects that were not fully appreciated at the time of launch (Rodgers et al., 2012). As a result current options for treatment are particularly limited, particularly in Europe where only Orlistat is available to prescribe and few people stick with taking it over the long term. Most forms of bariatric surgery have a relatively rapid and substantial beneficial effect on weight loss and health markers such as hyperglycaemia (Buchwald et al., 2009). However, the risk of side effects with bariatric surgery are substantially higher than all other medical treatments, with some researchers suggesting that if it were a medical treatment rather than a surgical one, it would by now have been halted by regulatory authorities, probably subject to appropriate controlled trials (Pinkney, 2011). The majority of surgeries are also carried out on severely obese patients (BMI > 45) and it is not clear that the same benefits experienced by this population would occur in those with less severe obesity (Pinkney, 2011).

There is therefore, currently a lack of successful obesity treatments. New approaches to aid weight and fat loss and prevent weight regain are therefore desirable. Due to the lack of success of pharmacological agents in providing a clear physiological benefit at an acceptable level of side effects, there is potential for naturally occurring bio-active ingredients that can produce positive effects on thermogenesis, fat oxidation and appetite to aid this process. Such compounds may be able to provide such an effect without the long-term problems (particularly on cardiovascular risk factors) associated with previously developed pharmacological agents (Yuliana et al., 2014). One such compound may be capsaicinoids, a compound naturally occurring in chillies (Whiting et al., 2012).

5.3.3 Capsaicinoids

Intervention trials evaluating the effects of capsaicinoids and their sister compounds capsinoids on weight loss outcomes suggest there may be an effect on 3 areas: increased energy expenditure, increased lipid oxidation and decreased energy intake. The results suggest that the effects are relatively modest, with figures of an increase in energy expenditure of around 50 kcal/day (Snitker et al., 2009, Galgani and Ravussin, 2010) and increase in lipid oxidation of around 20% (Lejeune et al., 2003) and a decrease in energy intake. Caution is however, required in interpreting these

results due to a number of reasons. Firstly, the majority of the research studies have been small scale intervention trials, with a low number of participants. This increases the likelihood of type 1 errors (finding an effect when there is not one) and type 2 errors (failing to find an effect when there is one) and are a major drawback of this area of research, with even the largest intervention trial having only 91 participants.

There are a number of ways in capsaicinoids and capsinoids may be providing a beneficial effect to weight loss management outcomes. Perhaps the most studied and well-established of these is the pathway through which energy expenditure is increased, via stimulation of the TRPV1 receptor and the resulting effect on catecholamine release signalling to adrenoceptors, which stimulate the SNS and cause uncoupling protein regulation resulting in enhanced energy expenditure (through thermogenesis), with BAT potentially playing a crucial role in this process. Research suggests capsaicinoids may also increase fat oxidation, with a number of potential mechanisms of this were outlined, namely SNS stimulation/catecholamine release, cellular TRPV1 stimulation, an effect on adipose tissue enzymes and protein expression and finally neuron stimulation. Less well established has been capsaicinoids potential effect on energy intake, which may be reduced following ingestion. This may be caused by capsaicinoids affecting intestinally derived satiety hormones, influencing certain areas of the brain, SNS stimulation, and increases in lipid oxidation. The effect on energy intake are less well established from intervention trial and therefore further investigation into these effects is warranted. To assess this further a meta-analysis into these effects was carried out.

5.3.4 Meta-Analysis into Capsaicinoids Effect on Energy Intake

The literature search carried out for the purposes of the meta-analysis suggest there has been 10 intervention trials carried out over the last 16 years investigating the effects of capsaicinoids on energy intake. However, results have been contradictory, with some trials observing a statistically significant effect (Westerterp-Plantenga et al., 2005) and others no effect (Smeets and Westerterp-Plantenga, 2009). In order to provide further evidence for the hypothesis that capsaicinoids may affect energy intake, a meta-analysis of intervention trials was undertaken.

Results from the meta-analysis into the effects of capsaicinoids on energy intake suggest there is a possibility they could play a role in weight management alongside exercise and other dietary measures. The combined effect size showed that consuming capsaicinoids caused a significant reduction in *ad libitum* energy intake of 251kJ (60kcal) per meal (see figure 3.2, for the forest plot), under the random effects model, with a 95% confidence interval of 337 – 166kJ and $p < 0.001$. The analysis indicated low heterogeneity, $I^2 = 6.6\%$. Results, however should be viewed with some caution as analysis indicates there was publication bias (p=0.007).

The results also suggest a dosage of a least 2mg of capsaicinoids is needed to produce an effect and that a reduction in energy intake may be the result of a preference for carbohydrate rich foods over fat rich foods. If the calculated reduction in energy intake (309.9kJ) was repeated on daily basis, weight loss modelling (Hall and Jordan, 2008) predicts the observed weight loss would be 5.3kg. After this the body's reduced calorie demands would result in weight maintenance (calculation based on sedentary male, 40 years of age, weighing 100kg). However, it should be noted such weight loss would take several years to achieve. The extent of the effect on energy intake is relatively small and therefore long term intake is likely to be required to produce a beneficial effect on weight management outcomes. Combined with capsaicinoids' effect on energy intake and lipid oxidation observed in other research (Ludy et al., 2012, Whiting et al., 2012) it maybe that capsaicinoids could produce a beneficial effect on body composition, especially body fat with a longer-term intervention. However, there had prior to this thesis been a lack of multi-week capsaicinoid interventions to investigate this hypothesis. To investigate the potential of capsaicinoids on body fat and other weight management outcomes further, an intervention trial was carried out.

5.3.5 Intervention Trial: Capsaicinoids and Body Fat in Caucasian Women

Reducing body fat mass is usually a key aim of diet and physical activity changes individuals undertake to as part of weight loss programs, although few capsaicinoid trials have assessed the potential effects. To the knowledge of the author, only two trials have assessed changes in body composition following a multi-week intervention with capsaicinoids (Lejeune et al., 2003, Ahuja et al., 2007). Change in body fat was

not the primary outcome in either study however and the research methodology of both trials means assessing change in body fat is difficult (see section 6.1.2 for more details). There have been around 30 trials, based around single dose interventions that have investigated the effects of capsaicinoids and capsinoids on energy expenditure, lipid oxidation and appetite. While findings from these trials are informative, to assess changes in body fat, longer-term trials are required. The present intervention trial was undertaken to test the hypothesis that capsaicinoids' effect energy expenditure, lipid oxidation and appetite could lead to a significant change in body fat with longer-term intervention.

Results from this research study suggest that supplementation with capsaicinoids may produce a small effect on body composition when ingested for six weeks, participants' body fat percentage and total body fat decreased by 0.64% and 0.67kg respectively. However, the robustness of this finding is called into question by the result of the interaction analysis which found no significant difference between the two groups over time for body adiposity. This decrease is also probably too small to provide any immediate health benefit. Therefore, supplementation would need to continue for a longer period and be done in conjunction with diet and exercise interventions. Typical targets for a health benefit from weight loss intervention are a reduction of 5-10% (Moyer, 2012), although there are no generally accepted targets for reductions in adipose tissue. There was no evidence of a statistically significant effect on body weight, following the supplementation period; however, excess adipose tissue storage, particularly for an extended period of time is a major health issue and reduction in levels could provide a powerful health benefit without weight reduction (Virtue and Vidal-Puig, 2010).

Reduction in white adipose tissue could provide a substantial health benefit to individuals, particularly those suffering from obesity. During recent years, research has found adipose tissue is an active endocrine organ that secretes more than 600 bioactive factors (Lehr et al., 2012). Of particular importance are adipokines (cell signalling proteins) play an important role in the regulation of appetite and satiety control, fat distribution and energy expenditure (Van Gaal et al., 2006). Altered adipokine secretion due to elevated adipose tissue levels has been associated with increased metabolic and cardiovascular obesity-related disorders (Blüher, 2013).

Reduction in adipose tissue levels should therefore be a key aim of obesity treatment and results from this trial suggest capsaicinoid supplementation may provide a benefit to individuals in aiding adipose tissue reduction.

Whether capsaicinoids can be of benefit in terms of actual body weight reduction is unclear from this research. There is some debate as to whether the types energy imbalance that capsaicinoids have been observed to cause (50-75kcal/day), could lead to long-term weight loss. It has been argued that the general increase in body weight observed in the United States over the past 3–4 decades, may have been triggered by a persistent positive energy imbalance of as little as 50 kcal/day (Hill, 2006). However, others have argued that 50kcal/day is just within the range of natural metabolic variability and would have minimal effect on weight loss over the long-term (Butte and Ellis, 2003). What is clear is that this trial was not long enough to definitively answer if capsaicinoid supplementation could lead to weight loss, and that a longer term trial of 1-2 years would be necessary to answer this question.

Also observed was a statistically significant reduction in mean fasting blood cholesterol levels of around 10%. Results of capsaicin interventions in animal models have been mixed (Zhang et al., 2013) and the one trial measuring effects of capsaicinoids in humans observed no effect following a four-week intervention diet (Ahuja et al., 2007). However the results observed in this trial raise the possibility that capsaicinoids may have a cholesterol lowering effect in humans, which is potentially of benefit to cardiovascular health (Goldstein and Brown, 2015), however further research is required to investigate this possibility further.

5.4 Conclusions

As stated in detail in section 2.5, obesity is a worldwide problem that threatens both the health of many individuals and the finances of many health services and economies (Wang et al., 2011). Levels may have peaked in certain developed economies, but are still growing in many parts of the world (Swinburn et al., 2011). As mentioned previously, treatment options are currently limited and either relatively unsuccessful (lifestyle modifications) or have serious side effects that may outweigh any benefit (pharmaceutical treatments and bariatric surgery). There is a strong need

for new treatment alternatives to be developed to work alongside current options and several bio-active compounds have shown initial promise in this area. However their development into potential weight loss and weight maintenance aids is unattractive for pharmaceutical companies; as natural occurring compounds they are not patentable and therefore they are not commercially viable for development and testing. The aim of this work therefore, was to look in detail at the potential for capsaicinoids to be used in this manner (see table 7.1 for the main aims and the resulting outcomes of the research project).

The present thesis found that a six-week capsaicinoid intervention could cause a significant reduction in body fat. However, the robustness of this finding was called into question by the results of the interaction analysis, which failed to find a significant difference between the placebo control and intervention groups over time. In addition, the reduction was small, and unlikely to be of significant clinical benefit for an individual's health. There was no evidence of a decrease in body weight following a six-week capsaicinoid intervention. For capsaicinoids to be of benefit in a real world setting, therefore a longer-term intervention period would be required, as effects are small and would need to slowly accumulate.

Capsaicinoid may also be have the potential to be of assistance in maintaining weight losses; long-term regain is a major issue for current obesity treatments. It has been established that in response to reduced calorific intake and weight loss, there is an accompanying decrease in energy expenditure, which is in part due to loss of lean body mass, and in part due to an enhanced metabolic efficiency (Major et al., 2007). Such reductions in thermogenesis may also persist well beyond the phase of weight loss (Rosenbaum et al., 2008) may make a significant contribution to weight regain and obesity relapse (Rosenbaum et al., 2008). Capsaicinoids potential to cause small increases in energy expenditure and fat oxidation, along with small decreases in energy intake may make them a suitable to aid the prevention of weight regain in individuals following calorie induced weight loss.

It is also likely that for meaningful weight management benefits, these compounds will need to be consumed as a supplement, rather than as a food, so individuals could adhere to the long-term intake required. Capsaicinoids have a pungent sensation

when eaten that many people find unpleasant and would stop them from regular consumption, which is likely to limit adherence to necessary ingestion rates. Therefore, supplementation may present the most realistic option for the long-term intake required to see a clinically significant benefit. Capsaicinoids, at the level used in this research, appear to be safe and were well tolerated, with minimal side effects for most participants. Suggesting they have the potential to be suitable for long-term supplementation, although further research is required. Capsinoids do not have the same pungency in the mouth and gastro-intestinal tract and may be of benefit to those who find consumption of capsaicinoids problematic. Overall there is promise in this area of research that capsaicinoids and capsinoids, may be a benefit for those looking to lose weight and to those looking to maintain losses already achieved.

Table 5.1 The main aims and outcomes of the research project

Aim	Date Performed	Key Findings	Outcome
Systematic literature search of human intervention trials investigating the effects of capsaicinoids and capsinoids on weight management outcomes	Initially: 07.10.2011 Updated: 14.10.2011	20 intervention trials into capsaicinoids and capsinoids effect on weight management outcomes in humans carried out since 1995	n/a
Collate and analyse findings of literature search and review their outcomes	07.2011 – 12.2011	50kcal/day increase in energy intake, a 20% increase in lipid oxidation and a decrease in energy intake.	Publication in Journal "Appetite" (see appendix 3)
Undertake a meta-analysis of the results of intervention trials measuring the effects of capsaicinoids on energy intake	01.2012 – 06.2013	A reduction in *ad-libitum* energy intake of 309.9kJ (74.0kcal) per meal, 95% confidence interval of 481.5 – 138.3kJ (115.0 – 33.0kcal) and $p < 0.001$	Publication in Journal "Appetite" (see appendix 3)
Undertake a double-blind, placebo controlled intervention trial to investigate the effects of capsaicinoids on body weight and composition in human subjects	02.2014-02.2016	A reduction in body fat percentage of 0.64% ($p = 0.022$) and in total body fat of 0.67kg ($p = 0.007$) after six weeks of capsaicinoids supplementation. No significant change in body weight observed.	Awaiting submission to journal

5.5 Recommendations for Further Work Based on this Research

There are a number of considerations to account for in the planning of future research in this area. Particularly informative in terms of the effect of capsaicinoids and capsinoids would be a long-term supplementation trial, lasting six months or longer. The nature of weight loss research necessitates long time periods of intervention as changes take time and there is a need for effects to be continued for the long term if interventions are to be effective. Also, despite much research in this area, observing changes in energy expenditure, lipid oxidation, energy intake and changes in body composition, no investigation has observed capsaicinoids or capsinoids aiding reductions in body weight. Any potential effect on weight loss appears to be small and trials have not been of sufficient length to conclude either affirmatively or negatively to this hypothesis.

It may be that if capsaicinoids or capsinoids were to be of benefit their effect would be best used in combination with other bio-active ingredients. Studies have investigated the effects of a number of other bio-active food compounds that may be of benefit to weight loss; in particular there are bodies of evidence for the effects of caffeine (Hursel and Westerterp-Plantenga, 2010) and catechins found in green tea (Phung et al., 2010). Bio-active ingredients have also been beneficially combined with other food ingredients such as inulin (Yang et al., 2012) and protein (Smeets et al., 2013). In addition to this, synergistic effects are sometimes observed when ingredients are combined and investigated (Shen et al., 2010, Zemel et al., 2004), which may occur in this instance. Generally, research involving multiple ingredient weight loss supplements including capsaicinoids or capsinoid has found a stronger effect than alone, however it is not clear if these ingredients are working synergistically. If a long-term trial weight loss trial was conducted in this area, with the financial investment that it would entail, it may be the best use of resources to investigate a multi-ingredient supplement.

The effect of capsaicinoids and capsinoids of differing ethnicities is also of interest. There are conflicting reports that there may be differing effects in differing ethnicities due to genetic adaptations that may have occurred over time (Hursel and Westerterp-Plantenga, 2010, Whiting et al., 2014). This is also a potential issue because of very

variable habitable dietary consumption rates of capsaicinoids in differing countries, ethnic groups and even within populations (Mattes, 2012). In addition to this, participants used for research trials have been either predominately Asian or Caucasian, meaning there is a particular lack of investigations in black populations. This may be an issue to be addressed in future research and should certainly be a consideration in planning.

There is evidence to suggest capsinoids may have a re-modelling effect on adipose tissue, turning stores of potentially harmful white adipose tissue into more beneficial brown or 'beige' adipose tissue (Yoneshiro et al., 2013). The functioning of adipose tissue is a relatively new area of research, with much still to be learnt. However initial investigations reveal some promise that harmful tissue can be altered in a beneficial way for health without major reductions in weight or body fat (Sun et al., 2011). Capsaicinoids and capsinoids may be compounds that can aid this process, and this promises to be an interesting area for future obesity research.

Also of potential interest would whether capsaicinoids and capsinoids can be of benefit when used with other lifestyle modifications to aid weight loss and/or maintenance. Changes to diet and physical activity combined with behaviour therapy typically produces weight loss in the region of 5-7% when applied for a relatively long period of time (e.g. six months) (Unick et al., 2011); however, as stated previously, weight regain is often an issue in this area (Strohacker et al., 2013). It would therefore be informative for the use of capsaicinoids and capsinoids in a non-research setting whether they can provide benefit by increasing initial body weight reductions and aiding maintenance of new weight level over the longer-term.

Following the observation that six weeks of capsaicinoid supplementation reduced fasting blood cholesterol, further research in this area is warranted. Previous research in animal models has observed mixed results (Zhang et al., 2013, Srinivasan and Chandrasekhara, 1992) an no statistically significant was observed in the only previous invention trial in humans (Ahuja et al., 2007). Of particular interest would be an investigation into capsaicinoids potential effect in those with elevated blood cholesterol and at increased risk of cardiovascular related health issues, as all

participants in this research had levels considered within the healthy range of values (Truswell, 2010).

Finally, in capsaicinoid and capsinoid weight loss trials, numerous differing dosages have been used; ranging from 0.4mg to 135mg/day (Lejeune et al., 2003, Reinbach et al., 2010). This is a large variation and is of concern particularly for the use of capsaicinoids, as they have a strong pungency and dosages that are too high may cause reductions in compliance. Meta-analysis work suggest effects occur and medium to high dosage levels (Ludy et al., 2012, Whiting et al., 2014), however while informative, this is far from conclusive. Choosing the correct dosage level is of vital importance and more research into the effects at different quantities would aid this.

References

ABRAMSON, J. 2011. WINPEPI updated: computer programs for epidemiologists, and their teaching potential. *Epidemiologic Perspectives & Innovations,* 8**,** 1.

ADAMS, M., AHUJA, K. & GERAGHTY, D. 2009. Effect of capsaicin and dihydrocapsaicin on< i> in vitro</i> blood coagulation and platelet aggregation. *Thrombosis research,* 124**,** 721-723.

ADAN, R., VANDERSCHUREN, L. & LA FLEUR, S. 2008. Anti-obesity drugs and neural circuits of feeding. *Trends in Pharmacological Sciences,* 29**,** 208-217.

ADES, P. & SAVAGE, P. The obesity paradox: perception vs knowledge. Mayo Clinic Proceedings, 2010. Mayo Foundation, 112.

AHMAD, F. & MAHMUD, S. 2010. Acute pancreatitis following orlistat therapy: report of two cases. *JOP,* 11**,** 61-3.

AHRÉN, B. 2004. Sensory nerves contribute to insulin secretion by glucagon-like peptide-1 in mice. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology,* 286**,** R269-R272.

AHUJA, K. & BALL, M. 2006. Effects of daily ingestion of chilli on serum lipoprotein oxidation in adult men and women. *British Journal of Nutrition,* 96**,** 239-242.

AHUJA, K., KUNDE, D., BALL, M. & GERAGHTY, D. 2006. Effects of capsaicin, dihydrocapsaicin, and curcumin on copper-induced oxidation of human serum lipids. *Journal of agricultural and food chemistry,* 54**,** 6436-6439.

AHUJA, K., ROBERTSON, I., GERAGHTY, D. & BALL, M. 2007. The effect of 4-week chilli supplementation on metabolic and arterial function in humans. *European journal of clinical nutrition,* 61**,** 326-33.

ALLENDER, S. & RAYNER, M. 2007. The burden of overweight and obesity‐related ill health in the UK. *Obesity reviews,* 8**,** 467-473.

ALLISON, D., GADDE, K., GARVEY, W., PETERSON, C., SCHWIERS, M., NAJARIAN, T., TAM, P., TROUPIN, B. & DAY, W. 2012. Controlled‐Release Phentermine/Topiramate in Severely Obese Adults: A Randomized Controlled Trial (EQUIP). *Obesity,* 20**,** 330-342.

AMBROSINI, G., DE KLERK, N., O'SULLIVAN, T., BEILIN, L. & ODDY, W. 2009. The reliability of a food frequency questionnaire for use among adolescents. *European journal of clinical nutrition,* 63**,** 1251-1259.

ANANDACOOMARASAMY, A., CATERSON, I., LEIBMAN, S., SMITH, G., SAMBROOK, P., FRANSEN, M. & MARCH, L. 2009. Influence of BMI on Health‐related Quality of Life: Comparison Between an Obese Adult Cohort and Age‐matched Population Norms. *Obesity,* 17**,** 2114-2118.

ANDERSON, L., JEFTINIJA, S., SCANES, C., STROMER, M., LEE, J., JEFTINIJA, K. & GLAVASKI-JOKSIMOVIC, A. 2005. Physiology of ghrelin and related peptides. *Domestic animal endocrinology,* 29**,** 111-144.

ARMSTRONG, L. 2006. *ACSM's guidelines for exercise testing and prescription/American College of*, Lippincott Williams & Wilkins, Philadelphia.

ARORA, R., GILL, N., CHAUHAN, G. & RANA, A. 2011. An Overview about Versatile Molecule Capsaicin. *International Journal of Pharmaceutical Sciences and Drug Research,* 3**,** 280-286.

ATLAS, D., STEER, M. & LEVITZKI, A. 1974. Stereospecific binding of propranolol and catecholamines to the β-adrenergic receptor. *Proceedings of the National Academy of Sciences,* 71**,** 4246-4248.

AUDELIN, M., SAVAGE, P. & ADES, P. 2008. Changing clinical profile of patients entering cardiac rehabilitation/secondary prevention programs: 1996 to 2006. *Journal of cardiopulmonary rehabilitation and prevention,* 28**,** 299-306.

AYONRINDE, O., OLYNYK, J., BEILIN, L., MORI, T., PENNELL, C., DE KLERK, N., ODDY, W., SHIPMAN, P. & ADAMS, L. 2011. Gender‐specific differences in adipose distribution and adipocytokines influence adolescent nonalcoholic fatty liver disease. *Hepatology,* 53**,** 800-809.

BABBAR, S., MARIER, J., MOUKSASSI, M., BELIVEAU, M., VANHOVE, G., CHANDA, S. & BLEY, K. 2009. Pharmacokinetic analysis of capsaicin after topical administration of a high-concentration capsaicin patch to patients with peripheral neuropathic pain. *Therapeutic drug monitoring,* 31**,** 502-510.

BABOOTA, R., MURTAZA, N., JAGTAP, S., SINGH, D., KARMASE, A., KAUR, J., BHUTANI, K., BOPARAI, R., PREMKUMAR, L. & KONDEPUDI, K. 2014. Capsaicin-induced transcriptional changes in hypothalamus and alterations in

gut microbial count in high fat diet fed mice. *The Journal of Nutritional Biochemistry*.

BARTE, J., TER BOGT, N., BOGERS, R., TEIXEIRA, P., BLISSMER, B., MORI, T. & BEMELMANS, W. 2010. Maintenance of weight loss after lifestyle interventions for overweight and obesity, a systematic review. *Obesity reviews,* 11**,** 899-906.

BARTNESS, T., DEMAS, G. & SONG, C. 2001. Central nervous system innervation of white adipose tissue. *Klaus S. Georgetown, TX: Landes Bioscience***,** 116-130.

BARTNESS, T., SHRESTHA, Y., VAUGHAN, C., SCHWARTZ, G. & SONG, C. 2010. Sensory and sympathetic nervous system control of white adipose tissue lipolysis. *Molecular and cellular endocrinology,* 318**,** 34-43.

BARTNESS, T. & SONG, C. 2007. Brain-adipose tissue neural crosstalk. *Physiology & Behavior,* 91**,** 343-351.

BARTNESS, T., SONG, C., SHI, H., BOWERS, R. & FOSTER, M. 2005. Brain–adipose tissue cross talk. *Proceedings of the Nutrition Society,* 64**,** 53-64.

BASTARD, J., MAACHI, M., LAGATHU, C., KIM, M., CARON, M., VIDAL, H., CAPEAU, J. & FEVE, B. 2006. Recent advances in the relationship between obesity, inflammation, and insulin resistance. *European cytokine network,* 17**,** 4-12.

BAX, L., YU, L., IKEDA, N., TSURUTA, H. & MOONS, K. 2006. Development and validation of MIX: comprehensive free software for meta-analysis of causal research data. *BMC medical research methodology,* 6**,** 50.

BAYLIE, R. & BRAYDEN, J. 2011. TRPV channels and vascular function. *Acta Physiologica,* 203**,** 99-116.

BAZZANO, L., GU, D., WHELTON, M., WU, X., CHEN, C., DUAN, X., CHEN, J., CHEN, J. & HE, J. 2010. Body mass index and risk of stroke among Chinese men and women. *Annals of neurology,* 67**,** 11-20.

BEN-MENACHEM, E., AXELSEN, M., JOHANSON, E., STAGGE, A. & SMITH, U. 2003. Predictors of Weight Loss in Adults with Topiramate Treated Epilepsy. *Obesity Research,* 11**,** 556-562.

BERGER, A., BRAND, M. & O'RAHILLY, S. 1998. Uncoupling proteins: the unravelling of obesity?: Increased understanding of mechanisms may lead, in time, to better drugs. *BMJ: British Medical Journal,* 317**,** 1607.

BERGMAN, R., STEFANOVSKI, D., BUCHANAN, T., SUMNER, A., REYNOLDS, J., SEBRING, N., XIANG, A. & WATANABE, R. 2011. A better index of body adiposity. *Obesity,* 19**,** 1083-9.

BERNARD, B., TSUBUKU, S., KAYAHARA, T., MAEDA, K., HAMADA, M., NAKAMURA, T., SHIRAI, Y., NAKAYAMA, A., UENO, S. & MIHARA, R. 2008.

Studies of the toxicological potential of capsinoids: X. Safety assessment and pharmacokinetics of capsinoids in healthy male volunteers after a single oral ingestion of CH-19 Sweet extract. *International journal of toxicology,* 27**,** 137-147.

BERTHOUD, H. 2008. Vagal and hormonal gut–brain communication: from satiation to satisfaction. *Neurogastroenterology & Motility,* 20**,** 64-72.

BERTHOUD, H. & MORRISON, C. 2008. The brain, appetite, and obesity. *Annu. Rev. Psychol.,* 59**,** 55-92.

BIGAARD, J., SPANGGAARD, I., THOMSEN, B., OVERVAD, K. & TJØNNELAND, A. 2005. Self-reported and technician-measured waist circumferences differ in middle-aged men and women. *The Journal of nutrition,* 135**,** 2263-2270.

BISHNOI, M., BOSGRAAF, C. & PREMKUMAR, L. 2011. Preservation of acute pain and efferent functions following intrathecal resiniferatoxin-induced analgesia in rats. *The Journal of Pain,* 12**,** 991-1003.

BLUNDELL, J. & COOLING, J. 2000. Routes to obesity: phenotypes, food choices and activity. *British Journal of Nutrition,* 83**,** S33-S38.

BLUNDELL, J., STUBBS, R., GOLDING, C., CRODEN, F., ALAM, R., WHYBROW, S., LE NOURY, J. & LAWTON, C. 2005. Resistance and susceptibility to weight gain: individual variability in response to a high-fat diet. *Physiology & Behavior,* 86**,** 614-622.

BLÜHER, M. 2013. Adipose tissue dysfunction contributes to obesity related metabolic diseases. *Best Practice & Research Clinical Endocrinology & Metabolism,* 27**,** 163-177.

BOOZER, C., DALY, P., HOMEL, P., SOLOMON, J., BLANCHARD, D., NASSER, J., STRAUSS, R. & MEREDITH, T. 2002. Herbal ephedra/caffeine for weight loss: a 6-month randomized safety and efficacy trial. *International journal of obesity and related metabolic disorders: journal of the International Association for the Study of Obesity,* 26**,** 593-604.

BOSLAND, P. 1992. Chiles: a diverse crop. *HortTechnology,* 2**,** 6-10.

BOSY-WESTPHAL, A., BOOKE, C.-A., BLÖCKER, T., KOSSEL, E., GOELE, K., LATER, W., HITZE, B., HELLER, M., GLÜER, C.-C. & MÜLLER, M. J. 2010. Measurement site for waist circumference affects its accuracy as an index of visceral and abdominal subcutaneous fat in a Caucasian population. *The Journal of nutrition,* 140**,** 954-961.

BROWNELL, K. 2004. *LEARN program for weight management,* Texas, American Health.

BUCHWALD, H., ESTOK, R., FAHRBACH, K., BANEL, D., JENSEN, M., PORIES, W., BANTLE, J. & SLEDGE, I. 2009. Weight and type 2 diabetes after bariatric

surgery: systematic review and meta-analysis. *The American journal of medicine,* 122**,** 248-256. e5.

BUCHWALD, H. & OIEN, D. 2013. Metabolic/bariatric surgery worldwide 2011. *Obesity surgery,* 23**,** 427-436.

BUFFENSTEIN, R., POPPITT, S. D., MCDEVITT, R. M. & PRENTICE, A. M. 1995. Food intake and the menstrual cycle: a retrospective analysis, with implications for appetite research. *Physiology & behavior,* 58**,** 1067-1077.

BUNT, J., LOHMAN, T. & BOILEAU, R. 1989. Impact of total body water fluctuations on estimation of body fat from body density. *Medicine and science in sports and exercise,* 21**,** 96-100.

BURGER, K. & BERNER, L. 2014. A functional neuroimaging review of obesity, appetitive hormones and ingestive behavior. *Physiology & Behavior*.

BURKE, L., WANG, J. & SEVICK, M. 2011. Self-monitoring in weight loss: a systematic review of the literature. *Journal of the American Dietetic Association,* 111**,** 92-102.

BUTTE, N. & ELLIS, K. 2003. Comment on" Obesity and the environment: where do we go from here?". *Science,* 301**,** 598-598.

BÁEZ, S., TSUCHIYA, Y., CALVO, A., PRUYAS, M., NAKAMURA, K., KIYOHARA, C., OYAMA, M. & YAMAMOTO, M. 2010. Genetic variants involved in gallstone formation and capsaicin metabolism, and the risk of gallbladder cancer in Chilean women. *World journal of gastroenterology: WJG,* 16**,** 372.

BÖHM, A. & HEITMANN, B. 2013. The use of bioelectrical impedance analysis for body composition in epidemiological studies. *European journal of clinical nutrition,* 67**,** S79-S85.

CADMUS-BERTRAM, L., WANG, J., PATTERSON, R., NEWMAN, V., PARKER, B. & PIERCE, J. 2013. Web‐based self‐monitoring for weight loss among overweight/obese women at increased risk for breast cancer: the HELP pilot study. *Psycho‐Oncology,* 22**,** 1821-1828.

CALLE, E. & KAAKS, R. 2004. Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. *Nature Reviews Cancer,* 4**,** 579-591.

CALLE, E., RODRIGUEZ, C., WALKER-THURMOND, K. & THUN, M. 2003. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of US adults. *New England Journal of Medicine,* 348**,** 1625-1638.

CAMHI, S., BRAY, G., BOUCHARD, C., GREENWAY, F., JOHNSON, W., NEWTON, R., RAVUSSIN, E., RYAN, D., SMITH, S. & KATZMARZYK, P. 2011. The relationship of waist circumference and BMI to visceral, subcutaneous, and total body fat: sex and race differences. *Obesity,* 19**,** 402-408.

CANNON, B. & NEDERGAARD, J. 2004. Brown adipose tissue: function and physiological significance. *Physiological reviews,* 84**,** 277-359.

CARVAJAL, R., WADDEN, T., TSAI, A., PECK, K. & MORAN, C. 2013. Managing obesity in primary care practice: a narrative review. *Annals of the New York Academy of Sciences,* 1281**,** 191-206.

CASANUEVA, B., RODERO, B., QUINTIAL, C., LLORCA, J. & GONZÁLEZ-GAY, M. 2013. Short-term efficacy of topical capsaicin therapy in severely affected fibromyalgia patients. *Rheumatology international,* 33**,** 2665-2670.

CATENACCI, V. A. & WYATT, H. R. 2007. The role of physical activity in producing and maintaining weight loss. *Nature Clinical Practice Endocrinology & Metabolism,* 3**,** 518-529.

CATERINA, M. J., SCHUMACHER, M. A., TOMINAGA, M., ROSEN, T. A., LEVINE, J. D. & JULIUS, D. 1997. The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature,* 389**,** 816-824.

CERIELLO, A., BARAKAT, M., BAHENDEKA, S., COLAGIURI, S., GERICH, J., HANEFELD, M., JI, L., LALIC, N., LEITER, L. A. & MONNIER, L. 2014. Guideline for management of postmeal glucose in diabetes. *DIABETES RESEARCH AND CLINICAL PRACTICE,* 103**,** 256-268.

CHANDA, S., MOULD, A., ESMAIL, A. & BLEY, K. 2005. Toxicity studies with pure trans-capsaicin delivered to dogs via intravenous administration. *Regulatory Toxicology and Pharmacology,* 43**,** 66-75.

CHUANG, W.-Y., KUNG, P.-H., KUO, C.-Y. & WU, C.-C. 2013. Sulforaphane prevents human platelet aggregation through inhibiting the phosphatidylinositol 3-kinase/Akt pathway. *Thrombosis & Haemostasis,* 109**,** 1120-1130.

CICHEWICZ, R. H. & THORPE, P. A. 1996. The antimicrobial properties of chile peppers (< i> Capsicum</i> species) and their uses in Mayan medicine. *Journal of ethnopharmacology,* 52**,** 61-70.

CINTI, S. 2007. The adipose organ. *Adipose tissue and adipokines in health and disease.* Springer.

CINTI, S. 2009. Transdifferentiation properties of adipocytes in the adipose organ. *American Journal of Physiology-Endocrinology And Metabolism,* 297**,** E977-E986.

CIOFFI, D. L. 2007. The skinny on TRPV1. *Circulation research,* 100**,** 934-936.

CISNEROS-PINEDA, O., TORRES-TAPIA, L. W., GUTIÉRREZ-PACHECO, L. C., CONTRERAS-MARTÍN, F., GONZÁLEZ-ESTRADA, T. & PERAZA-SÁNCHEZ, S. R. 2007. Capsaicinoids quantification in chili peppers cultivated in the state of Yucatan, Mexico. *Food Chemistry,* 104**,** 1755-1760.

COLDITZ, G. A., WILLETT, W. C., ROTNITZKY, A. & MANSON, J. E. 1995. Weight gain as a risk factor for clinical diabetes mellitus in women. *Annals of internal medicine,* 122**,** 481-486.

CONSORTIUM, L. 2009. Peri-operative safety in the longitudinal assessment of bariatric surgery. *The New England journal of medicine,* 361**,** 445.

CORDELL, G. A. & ARAUJO, O. E. 1993. Capsaicin: identification, nomenclature, and pharmacotherapy. *The Annals of pharmacotherapy,* 27**,** 330-336.

COUSIN, B., CASTEILLA, L., LAFONTAN, M., AMBID, L., LANGIN, D., BERTHAULT, M. & PENICAUD, L. 1993. Local sympathetic denervation of white adipose tissue in rats induces preadipocyte proliferation without noticeable changes in metabolism. *Endocrinology,* 133**,** 2255-2262.

COX, H. M., TOUGH, I. R., WOOLSTON, A.-M., ZHANG, L., NGUYEN, A. D., SAINSBURY, A. & HERZOG, H. 2010. Peptide YY is critical for acylethanolamine receptor Gpr119-induced activation of gastrointestinal mucosal responses. *Cell metabolism,* 11**,** 532-542.

CURIONI, C. C. & LOURENCO, P. M. 2005. Long-term weight loss after diet and exercise: a systematic review. *Int J Obes Relat Metab Disord,* 29**,** 1168-1174.

DALCANALE, L., OLIVEIRA, C. P., FAINTUCH, J., NOGUEIRA, M. A., RONDÓ, P., LIMA, V. M., MENDONÇA, S., PAJECKI, D., MANCINI, M. & CARRILHO, F. J. 2010. Long-term nutritional outcome after gastric bypass. *Obesity surgery,* 20**,** 181-187.

DANG, D. K. 2012. Helping patients with diabetes and visual or manual impairment (part 2): Medication administration.

DATTA, S. & JANA, J. 2010. Genetic variability, heritability and correlation in chilli genotypes under Terai zone of West Bengal. *SAARC Journal of Agriculture,* 8**,** 33-45.

DAVIS, C., MARKEY, C., BUSCH, M. & BUSCH, K. 2007. Determination of capsaicinoids in habanero peppers by chemometric analysis of UV spectral data. *Journal of agricultural and food chemistry,* 55**,** 5925-5933.

DE PETROCELLIS, L., ORLANDO, P., MORIELLO, A. S., AVIELLO, G., STOTT, C., IZZO, A. & DI MARZO, V. 2012. Cannabinoid actions at TRPV channels: effects on TRPV3 and TRPV4 and their potential relevance to gastrointestinal inflammation. *Acta Physiologica,* 204**,** 255-266.

DEBRECENI, A., ABDEL-SALAM, O. M., FIGLER, M., JURICSKAY, I., SZOLCSÁNYI, J. & MÓZSIK, G. 1999. Capsaicin increases gastric emptying rate in healthy human subjects measured by 13 C-labeled octanoic acid breath test. *Journal of Physiology-Paris,* 93**,** 455-460.

DELILLE, J. & RAMIREZ, E. 1935. Pharmacodynamic action of the active principles of chile (capsicum annum L.) Anales Inst. *Biology,* 6**,** 23-37.

DEMARIA, E. J., PATE, V., WARTHEN, M. & WINEGAR, D. A. 2010. Baseline data from American society for metabolic and bariatric surgery-designated bariatric surgery centers of excellence using the bariatric outcomes longitudinal database. *Surgery for Obesity and Related Diseases,* 6**,** 347-355.

DEURENBERG, P., ANDREOLI, A., BORG, P., KUKKONEN-HARJULA, K., DE LORENZO, A., VAN MARKEN, L. W., TESTOLIN, G., VIGANO, R. & VOLLAARD, N. 2001. The validity of predicted body fat percentage from body mass index and from impedance in samples of five European populations. *European journal of clinical nutrition,* 55**,** 973-979.

DEURENBERG, P., WESTSTRATE, J. & SEIDELL, J. 1991. Body mass index as a measure of body fatness: age-and sex-specific prediction formulas. *British Journal of Nutrition,* 65**,** 105-114.

DICPINIGAITIS, P. V. & ALVA, R. V. 2005. Safety of capsaicin cough challenge testing. *CHEST Journal,* 128**,** 196-202.

DIETRICH, M. O. & HORVATH, T. L. 2013. Hypothalamic control of energy balance: insights into the role of synaptic plasticity. *Trends in neurosciences,* 36**,** 65-73.

DIETZ, W. H. 2015. The Response of the US Centers for Disease Control and Prevention to the Obesity Epidemic. *Annual review of public health,* 36**,** 575-596.

DIXON, J. B., STRAZNICKY, N. E., LAMBERT, E. A., SCHLAICH, M. P. & LAMBERT, G. W. 2012. Laparoscopic adjustable gastric banding and other devices for the management of obesity. *Circulation,* 126**,** 774-785.

DOAK, C. M., ADAIR, L. S., BENTLEY, M., MONTEIRO, C. & POPKIN, B. M. 2005. The dual burden household and the nutrition transition paradox. *International journal of obesity,* 29**,** 129-136.

DONNELLY, J. E., BLAIR, S., JAKICIC, J., MANORE, M., RANKIN, J. & SMITH, B. 2009. American College of Sports Medicine Position Stand. Appropriate physical activity intervention strategies for weight loss and prevention of weight regain for adults. *Medicine and science in sports and exercise,* 41**,** 459-471.

DONNERER, J., AMANN, R., SCHULIGOI, R. & LEMBECK, F. 1990. Absorption and metabolism of capsaicinoids following intragastric administration in rats. *Naunyn-Schmiedeberg's archives of pharmacology,* 342**,** 357-361.

DOUKETIS, J., MACIE, C., THABANE, L. & WILLIAMSON, D. 2005. Systematic review of long-term weight loss studies in obese adults: clinical significance and applicability to clinical practice. *International journal of obesity,* 29**,** 1153-1167.

DULLOO, A., SEYDOUX, J. & JACQUET, J. 2004. Adaptive thermogenesis and uncoupling proteins: a reappraisal of their roles in fat metabolism and energy balance. *Physiology & Behavior,* 83**,** 587-602.

DÍAZ, J., POMAR, F., BERNAL, A. & MERINO, F. 2004. Peroxidases and the metabolism of capsaicin in Capsicum annuum L. *Phytochemistry Reviews,* 3**,** 141-157.

EVELETH, P. B. & TANNER, J. J. M. 1976. *World-wide variation in human growth*, CUP Archive.

EZZATI, M., VANDER HOORN, S., LAWES, C. M., LEACH, R., JAMES, W. P. T., LOPEZ, A. D., RODGERS, A. & MURRAY, C. J. 2005. Rethinking the "diseases of affluence" paradigm: global patterns of nutritional risks in relation to economic development. *PLoS medicine,* 2**,** e133.

FAUL, F., ERDFELDER, E., BUCHNER, A. & LANG, A. 2009. Statistical power analyses using G* Power 3.1: Tests for correlation and regression analyses. *Behavior research methods,* 41**,** 1149-1160.

FIDLER, M. C., SANCHEZ, M., RAETHER, B., WEISSMAN, N. J., SMITH, S. R., SHANAHAN, W. R. & ANDERSON, C. M. 2011. A one-year randomized trial of lorcaserin for weight loss in obese and overweight adults: the BLOSSOM trial. *The Journal of Clinical Endocrinology & Metabolism,* 96**,** 3067-3077.

FILIPPATOS, T. D., DERDEMEZIS, C. S., GAZI, I. F., NAKOU, E. S., MIKHAILIDIS, D. P. & ELISAF, M. S. 2008. Orlistat-associated adverse effects and drug interactions. *Drug Safety,* 31**,** 53-65.

FINGER, F., LANNES, S., SCHUELTER, A., DOEGE, J., COMERLATO, A., GONÇALVES, L., FERREIRA, F., CLOVIS, L. & SCAPIM, C. 2010. Genetic diversity of Capsicum chinensis (Solanaceae) accessions based on molecular markers and morphological and agronomic traits. *Genetics and Molecular Research,* 9**,** 1852-1864.

FINKELSTEIN, E. A., DACOSTA DIBONAVENTURA, M., BURGESS, S. M. & HALE, B. C. 2010. The costs of obesity in the workplace. *Journal of Occupational and Environmental Medicine,* 52**,** 971-976.

FINKELSTEIN, E. A., TROGDON, J. G., COHEN, J. W. & DIETZ, W. 2009. Annual medical spending attributable to obesity: payer-and service-specific estimates. *Health affairs,* 28**,** w822-w831.

FINUCANE, M. M., STEVENS, G. A., COWAN, M. J., DANAEI, G., LIN, J. K., PACIOREK, C. J., SINGH, G. M., GUTIERREZ, H. R., LU, Y. & BAHALIM, A. N. 2011. National, regional, and global trends in body-mass index since 1980: systematic analysis of health examination surveys and epidemiological studies with 960 country-years and 9· 1 million participants. *The Lancet,* 377**,** 557-567.

FLEGAL, K. M., CARROLL, M. D., KIT, B. K. & OGDEN, C. L. 2012. Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999-2010. *JAMA : the journal of the American Medical Association,* 307**,** 491-497.

FLEGAL, K. M., GRAUBARD, B. I., WILLIAMSON, D. F. & GAIL, M. H. 2005. Excess deaths associated with underweight, overweight, and obesity. *JAMA : the journal of the American Medical Association,* 293**,** 1861-1867.

FONTAINE, K. & BAROFSKY, I. 2001. Obesity and health‐related quality of life. *Obesity reviews,* 2**,** 173-182.

FOSTER, G. D., BORRADAILE, K. E., SANDERS, M. H., MILLMAN, R., ZAMMIT, G., NEWMAN, A. B., WADDEN, T. A., KELLEY, D., WING, R. R. & PI-SUNYER, F. X. 2009. A randomized study on the effect of weight loss on obstructive sleep apnea among obese patients with type 2 diabetes: the Sleep AHEAD study. *Archives of internal medicine,* 169**,** 1619-1626.

FOSTER, G. D., WYATT, H. R., HILL, J. O., MAKRIS, A. P., ROSENBAUM, D. L., BRILL, C., STEIN, R. I., MOHAMMED, B. S., MILLER, B. & RADER, D. J. 2010. Weight and metabolic outcomes after 2 years on a low-carbohydrate versus low-fat dietA randomized trial. *Annals of internal medicine,* 153**,** 147-157.

FU-CHENG, X., ANINI, Y., CHARIOT, J., CASTEX, N., GALMICHE, J.-P. & ROZE, C. 1997. Mechanisms of peptide YY release induced by an intraduodenal meal in rats: neural regulation by proximal gut. *Pflügers Archiv,* 433**,** 571-579.

FUGH-BERMAN, A. 2000. Herb-drug interactions. *The Lancet,* 355**,** 134-138.

FUJIOKA, K., DIBAISE, J. K. & MARTINDALE, R. G. 2011. Nutrition and metabolic complications after bariatric surgery and their treatment. *Journal of Parenteral and Enteral Nutrition,* 35**,** 52S-59S.

FUJIWAKE, H., SUZUKI, T., OKA, S. & IWAI, K. 1980. Enzymatic formation of capsaicinoid from vanillylamine and iso-type fatty acids by cell-free extracts of Capsicum annuum var. annuum cv. Karayatsubusa. *Agricultural and biological chemistry*.

FUKUDA, H., MIZUTA, Y., ISOMOTO, H., TAKESHIMA, F., OHNITA, K., OHBA, K., OMAGARI, K., TANIYAMA, K. & KOHNO, S. 2004. Ghrelin enhances gastric motility through direct stimulation of intrinsic neural pathways and capsaicin-sensitive afferent neurones in rats. *Scandinavian journal of gastroenterology,* 39**,** 1209-1214.

FURIE, K. L., KASNER, S. E., ADAMS, R. J., ALBERS, G. W., BUSH, R. L., FAGAN, S. C., HALPERIN, J. L., JOHNSTON, S. C., KATZAN, I. & KERNAN, W. N. 2011. Guidelines for the prevention of stroke in patients with stroke or transient ischemic attack a guideline for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke,* 42**,** 227-276.

FUSTER, V. & KELLY, B. B. 2010. *Promoting cardiovascular health in the developing world: a critical challenge to achieve global health*, National Academies Press.

GADDE, K. M., ALLISON, D. B., RYAN, D. H., PETERSON, C. A., TROUPIN, B., SCHWIERS, M. L. & DAY, W. W. 2011. Effects of low-dose, controlled-release,

phentermine plus topiramate combination on weight and associated comorbidities in overweight and obese adults (CONQUER): a randomised, placebo-controlled, phase 3 trial. *The Lancet,* 377**,** 1341-1352.

GALGANI, J. E. & RAVUSSIN, E. 2010. Effect of dihydrocapsiate on resting metabolic rate in humans. *The American journal of clinical nutrition,* 92**,** 1089-93.

GALIC, S., OAKHILL, J. S. & STEINBERG, G. R. 2010. Adipose tissue as an endocrine organ. *Molecular and cellular endocrinology,* 316**,** 129-139.

GARAMI, A., BALASKÓ, M., SZÉKELY, M., SOLYMÁR, M. & PÉTERVÁRI, E. 2010. Fasting hypometabolism and refeeding hyperphagia in rats: Effects of capsaicin desensitization of the abdominal vagus. *European journal of pharmacology,* 644**,** 61-66.

GARBER, A. M. & PHELPS, C. E. 1997. Economic foundations of cost-effectiveness analysis. *Journal of health economics,* 16**,** 1-31.

GARDIN, J. M., SCHUMACHER, D., CONSTANTINE, G., DAVIS, K. D., LEUNG, C. & REID, C. L. 2000. Valvular abnormalities and cardiovascular status following exposure to dexfenfluramine or phentermine/fenfluramine. *JAMA : the journal of the American Medical Association,* 283**,** 1703-1709.

GARG, S. K., MAURER, H., REED, K. & SELAGAMSETTY, R. 2014. Diabetes and cancer: two diseases with obesity as a common risk factor. *Diabetes, Obesity and Metabolism,* 16**,** 97-110.

GARROW, J. & SUMMERBELL, C. 1995. Meta-analysis: effect of exercise, with or without dieting, on the body composition of overweight subjects. *European journal of clinical nutrition,* 49**,** 1-10.

GAVVA, N. R., BANNON, A. W., SURAPANENI, S., HOVLAND, D. N., LEHTO, S. G., GORE, A., JUAN, T., DENG, H., HAN, B. & KLIONSKY, L. 2007. The vanilloid receptor TRPV1 is tonically activated in vivo and involved in body temperature regulation. *The Journal of Neuroscience,* 27**,** 3366-3374.

GEER, E. B. & SHEN, W. 2009. Gender differences in insulin resistance, body composition, and energy balance. *Gender medicine,* 6**,** 60-75.

GERNER, P., BINSHTOK, A. M., WANG, C.-F., HEVELONE, N. D., BEAN, B. P., WOOLF, C. J. & WANG, G. K. 2008. Capsaicin combined with local anesthetics preferentially prolongs sensory/nociceptive block in rat sciatic nerve. *Anesthesiology,* 109**,** 872.

GHARAT, L. & SZALLASI, A. 2007. Medicinal chemistry of the vanilloid (Capsaicin) TRPV1 receptor: Current knowledge and future perspectives. *Drug Development Research,* 68**,** 477-497.

GOLDSTEIN, J. & BROWN, M. 2015. A century of cholesterol and coronaries: from plaques to genes to statins. *Cell,* 161**,** 161-172.

GOLDSTEIN, L., BUSHNELL, C., ADAMS, R., APPEL, L., BRAUN, L., CHATURVEDI, S., CREAGER, M., CULEBRAS, A., ECKEL, R. & HART, R. 2011. Guidelines for the Primary Prevention of Stroke A Guideline for Healthcare Professionals From the American Heart Association/American Stroke Association. *Stroke,* 42**,** 517-584.

GONLACHANVIT, S. 2010. Are rice and spicy diet good for functional gastrointestinal disorders? *Journal of neurogastroenterology and motility,* 16**,** 131-8.

GORTMAKER, S. L., SWINBURN, B. A., LEVY, D., CARTER, R., MABRY, P. L., FINEGOOD, D. T., HUANG, T., MARSH, T. & MOODIE, M. L. 2011. Changing the future of obesity: science, policy, and action. *The Lancet,* 378**,** 838-847.

GREENHOUSE, S. & GEISSER, S. 1959. On methods in the analysis of profile data. *Psychometrika,* 24**,** 95-112.

GRUNDY, S. M., CLEEMAN, J. I., DANIELS, S. R., DONATO, K. A., ECKEL, R. H., FRANKLIN, B. A., GORDON, D. J., KRAUSS, R. M., SAVAGE, P. J. & SMITH, S. C. 2005. Diagnosis and management of the metabolic syndrome an American Heart Association/National Heart, Lung, and Blood Institute scientific statement. *Circulation,* 112**,** 2735-2752.

GUH, D. P., ZHANG, W., BANSBACK, N., AMARSI, Z., BIRMINGHAM, C. L. & ANIS, A. H. 2009. The incidence of co-morbidities related to obesity and overweight: a systematic review and meta-analysis. *BMC public health,* 9**,** 88.

GUZZARDI, M. A. & IOZZO, P. 2013. Obesity and Diabetes. *Interdisciplinary Concepts in Cardiovascular Health.* Springer.

HALL, K. D. & JORDAN, P. N. 2008. Modeling weight-loss maintenance to help prevent body weight regain. *American Journal of Clinical Nutrition,* 88**,** 1495-1503.

HAMPP, C., KANG, E. M. & BORDERS‐HEMPHILL, V. 2013. Use of Prescription Antiobesity Drugs in the United States. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy,* 33**,** 1299-1307.

HARADA, N. & OKAJIMA, K. 2009. Effects of capsaicin and isoflavone on blood pressure and serum levels of insulin-like growth factor-I in normotensive and hypertensive volunteers with alopecia. *Bioscience, biotechnology, and biochemistry,* 73**,** 1456-1459.

HAWKES, C. 2006. Uneven dietary development: linking the policies and processes of globalization with the nutrition transition, obesity and diet-related chronic diseases. *Globalization and health,* 2**,** 4.

HAYMAN, M. & KAM, P. C. 2008. Capsaicin: A review of its pharmacology and clinical applications. *Current Anaesthesia & Critical Care,* 19**,** 338-343.

HE, G.-J., YE, X.-L., MOU, X., CHEN, Z. & LI, X.-G. 2009. Synthesis and antinociceptive activity of capsinoid derivatives. *European journal of medicinal chemistry,* 44**,** 3345-3349.

HENRY, C. 2005. Basal metabolic rate studies in humans: measurement and development of new equations. *Public health nutrition,* 8**,** 1133-1152.

HENRY, C. & EMERY, B. 1986. Effect of spiced food on metabolic rate. *Human nutrition. Clinical nutrition,* 40**,** 165-168.

HILL, J. O. 2006. Understanding and addressing the epidemic of obesity: an energy balance perspective. *Endocrine reviews,* 27**,** 750-61.

HILLE, B. 2001. *Ion channels of excitable membranes*, Sinauer Sunderland, MA.

HOLST, J. J. 2007. The physiology of glucagon-like peptide 1. *Physiological reviews,* 87**,** 1409-1439.

HOLZER, P. 2008. The pharmacological challenge to tame the transient receptor potential vanilloid‐1 (TRPV1) nocisensor. *British journal of pharmacology,* 155**,** 1145-1162.

HSU, C. & YEN, G. 2007. Effects of capsaicin on induction of apoptosis and inhibition of adipogenesis in 3T3-L1 cells. *Journal of agricultural and food chemistry,* 55**,** 1730-1736.

HURSEL, R. & WESTERTERP-PLANTENGA, M. 2010. Thermogenic ingredients and body weight regulation. *International journal of obesity,* 34**,** 659-69.

IBM CORP. 2013. IBM SPSS Statistics for Windows. 22.0 ed. Armonk, NY: IBM Corp.

IIDA, T., MORIYAMA, T., KOBATA, K., MORITA, A., MURAYAMA, N., HASHIZUME, S., FUSHIKI, T., YAZAWA, S., WATANABE, T. & TOMINAGA, M. 2003. TRPV1 activation and induction of nociceptive response by a non-pungent capsaicin-like compound, capsiate. *Neuropharmacology,* 44**,** 958-67.

IONUT, V., BURCH, M., YOUDIM, A. & BERGMAN, R. N. 2013. Gastrointestinal hormones and bariatric surgery‐induced weight loss. *Obesity,* 21**,** 1093-1103.

ITO, N., RUEGG, U., KUDO, A., MIYAGOE-SUZUKI, Y. & TAKEDA, S. 2013. Capsaicin mimics mechanical load-induced intracellular signaling events. *Channels,* 7**,** 221-224.

JAMES, W. 2008. WHO recognition of the global obesity epidemic. *International journal of obesity,* 32**,** S120-S126.

JANSSENS, P., HURSEL, R., MARTENS, E. & WESTERTERP-PLANTENGA, M. 2013. Acute effects of capsaicin on energy expenditure and fat oxidation in negative energy balance. *PloS one,* 8**,** e67786.

JANSSENS, P., HURSEL, R. & WESTERTERP-PLANTENGA, M. 2014. Capsaicin increases sensation of fullness in energy balance, and decreases desire to eat after dinner in negative energy balance. *Appetite,* 77**,** 46-51.

JANSSENS, P., VANDEN-BERGHE, P., VERSCHUEREN, S., LEHMANN, A., DEPOORTERE, I. & TACK, J. 2011. Review article: the role of gastric motility in the control of food intake. *Alimentary pharmacology & therapeutics,* 33**,** 880-894.

JIANG, X., JIA, L.-W., LI, X.-H., CHENG, X.-S., XIE, J.-Z., MA, Z.-W., XU, W.-J., LIU, Y., YAO, Y. & DU, L.-L. 2013. Capsaicin ameliorates stress-induced Alzheimer's disease-like pathological and cognitive impairments in rats. *Journal of Alzheimer's Disease,* 35**,** 91-105.

JOO, J. I., KIM, D. H., CHOI, J.-W. & YUN, J. W. 2010. Proteomic analysis for antiobesity potential of capsaicin on white adipose tissue in rats fed with a high fat diet. *Journal of proteome research,* 9**,** 2977-2987.

JOSSE, A. R., SHERRIFFS, S. S., HOLWERDA, A. M., ANDREWS, R., STAPLES, A. W. & PHILLIPS, S. M. 2010. Effects of capsinoid ingestion on energy expenditure and lipid oxidation at rest and during exercise. *Nutrition & metabolism,* 7**,** 65.

JULIUS, D. & BASBAUM, A. I. 2001. Molecular mechanisms of nociception. *Nature,* 413**,** 203-210.

KADOHISA, M., ROLLS, E. T. & VERHAGEN, J. V. 2004. Orbitofrontal cortex: neuronal representation of oral temperature and capsaicin in addition to taste and texture. *Neuroscience,* 127**,** 207-21.

KAPTCHUK, T. J. & MILLER, F. G. 2015. Placebo effects in medicine. *New England Journal of Medicine,* 373**,** 8-9.

KAWABATA, F., INOUE, N., MASAMOTO, Y., MATSUMURA, S., KIMURA, W., KADOWAKI, M., HIGASHI, T., TOMINAGA, M., INOUE, K. & FUSHIKI, T. 2009. Non-pungent capsaicin analogs (capsinoids) increase metabolic rate and enhance thermogenesis via gastrointestinal TRPV1 in mice. *Bioscience, biotechnology, and biochemistry,* 73**,** 2690-7.

KAWABATA, F., INOUE, N., YAZAWA, S., KAWADA, T., INOUE, K. & FUSHIKI, T. 2006. Effects of CH-19 sweet, a non-pungent cultivar of red pepper, in decreasing the body weight and suppressing body fat accumulation by sympathetic nerve activation in humans. *Bioscience, biotechnology, and biochemistry,* 70**,** 2824-35.

KAWADA, T., SAKABE, S., WATANABE, T., YAMAMOTO, M. & IWAI, K. 1988. Some pungent principles of spices cause the adrenal medulla to secrete catecholamine in anesthetized rats. *Experimental Biology and Medicine,* 188**,** 229-233.

KAWADA, T., SUZUKI, T., TAKAHASHI, M. & IWAI, K. 1984. Gastrointestinal absorption and metabolism of capsaicin and dihydrocapsaicin in rats. *Toxicology and applied pharmacology,* 72**,** 449-456.

KAWADA, T., WATANABE, T., TAKAISHI, T., TANAKA, T. & IWAI, K. 1986. Capsaicin-induced β-adrenergic action on energy metabolism in rats: influence of capsaicin on oxygen consumption, the respiratory quotient, and substrate utilization. *Experimental Biology and Medicine,* 183**,** 250-256.

KEARNEY, J. 2010. Food consumption trends and drivers. *Philosophical transactions of the royal society B: biological sciences,* 365**,** 2793-2807.

KELLY, T., YANG, W., CHEN, C., REYNOLDS, K. & HE, J. 2008. Global burden of obesity in 2005 and projections to 2030. *International journal of obesity,* 32**,** 1431-1437.

KEOGH, J. B. & CLIFTON, P. M. 2005. The role of meal replacements in obesity treatment. *Obesity reviews,* 6**,** 229-234.

KIM, S., HWANG, J., PARK, H., KWON, D. & KIM, M. 2013. Capsaicin stimulates glucose uptake in C2C12 muscle cells via the reactive oxygen species (ROS)/AMPK/p38 MAPK pathway. *Biochemical and biophysical research communications,* 439**,** 66-70.

KIRCHENGAST, S. & GARTNER, M. 2002. Changes in fat distribution (WHR) and body weight across the menstrual cycle. *Collegium Antropologicum,* 26**,** 47-57.

KNOTKOVA, H., PAPPAGALLO, M. & SZALLASI, A. 2008. Capsaicin (TRPV1 Agonist) therapy for pain relief: farewell or revival? *The Clinical journal of pain,* 24**,** 142-54.

KNOWLER, W. C., BARRETT-CONNOR, E., FOWLER, S. E., HAMMAN, R. F., LACHIN, J. M., WALKER, E. A. & NATHAN, D. M. 2002. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *The New England journal of medicine,* 346**,** 393-403.

KOBATA, K., SUTOH, K., TODO, T., YAZAWA, S., IWAI, K. & WATANABE, T. 1999. Nordihydrocapsiate, a new capsinoid from the fruits of a nonpungent pepper, capsicum annuum. *Journal of natural products,* 62**,** 335-6.

KOBATA, K., TODO, T., YAZAWA, S., IWAI, K. & WATANABE, T. 1998. Novel capsaicinoid-like substances, capsiate and dihydrocapsiate, from the fruits of a nonpungent cultivar, CH-19 Sweet, of pepper (Capsicum annuum L.). *Journal of agricultural and food chemistry,* 46**,** 1695-1697.

KODAMA, T., MASUYAMA, T., KAYAHARA, T., TSUBUKU, S., OHISHI, T., WAGNER, B. M. & BERNARD, B. K. 2010. Studies of the Toxicological Potential of Capsinoids: A 26-week Gavage Toxicity Study of Dihydrocapsiate in Rats. *International journal of toxicology,* 29**,** 27S-54S.

KOSUGE, S., INAGAKI, Y. & OKUMURA, H. 1961. Studies on the pungent principles of red pepper. *Part VIII. On the chemical constitutions of the pungent principles. Nippon Nogei Kagaku Kaishi (J. Agric. Chem. Soc.),* 35**,** 923-927.

KUMAR, S., MONIF, T., ARORA, R., KHUROO, A., JAIN, R., REYAR, S., VERMA, P. R. P. & RAO, S. 2013. The pharmacodynamic equivalence of Orlistat 60 mg capsule. An open label, balanced, randomized, multiple-dose, cross-over pharmacodynamic end-point bioequivalence study in healthy, adult, human Asian Indian subjects under fed conditions. *Clinical Research and Regulatory Affairs,* 30**,** 55-62.

KUSHNER, R. F. 2008. Anti-obesity drugs. *Expert opinion on pharmacotherapy,* 9**,** 1339-1350.

KUSHNER, R. F. 2013. Weight Loss Strategies for Treatment of Obesity. *Progress in cardiovascular diseases*.

KUSMINSKI, C. M. & SCHERER, P. E. 2012. Mitochondrial dysfunction in white adipose tissue. *Trends in endocrinology & metabolism,* 23**,** 435-443.

LAFERRÈRE, B., REILLY, D., ARIAS, S., SWERDLOW, N., GORROOCHURN, P., BAWA, B., BOSE, M., TEIXEIRA, J., STEVENS, R. D. & WENNER, B. R. 2011. Differential metabolic impact of gastric bypass surgery versus dietary intervention in obese diabetic subjects despite identical weight loss. *Science translational medicine,* 3**,** 80re2-80re2.

LATIMER, G. W. 2012. *Official methods of Analysis of AOAC International*, AOAC International.

LAUER, M. S. 2012. Lemons for obesity. *Annals of internal medicine,* 157**,** 139-140.

LE ROUX, C. W., WELBOURN, R., WERLING, M., OSBORNE, A., KOKKINOS, A., LAURENIUS, A., LÖNROTH, H., FÄNDRIKS, L., GHATEI, M. A. & BLOOM, S. R. 2007. Gut hormones as mediators of appetite and weight loss after Roux-en-Y gastric bypass. *Annals of surgery,* 246**,** 780-785.

LEATHERDALE, S. T. & LAXER, R. E. 2013. Reliability and validity of the weight status and dietary intake measures in the COMPASS questionnaire: are the self-reported measures of body mass index (BMI) and Canada's food guide servings robust? *Int J Behav Nutr Phys Act,* 10**,** 42.

LEE, C., COLAGIURI, S., EZZATI, M. & WOODWARD, M. 2011. The burden of cardiovascular disease associated with high body mass index in the Asia–Pacific region. *Obesity reviews,* 12**,** e454-e459.

LEE, P., GREENFIELD, J., HO, K. & FULHAM, M. 2010a. A critical appraisal of the prevalence and metabolic significance of brown adipose tissue in adult humans. *American Journal of Physiology-Endocrinology And Metabolism,* 299**,** E601-E606.

LEE, R. 2008. Future costs in cost effectiveness analysis. *Journal of health economics,* 27**,** 809-818.

LEE, T., LI, Z., ZERLIN, A. & HEBER, D. 2010b. Effects of dihydrocapsiate on adaptive and diet-induced thermogenesis with a high protein very low calorie diet: a randomized control trial. *Nutrition & metabolism,* 7**,** 78.

LEHNERT, T., SONNTAG, D., KONNOPKA, A., RIEDEL-HELLER, S. & KÖNIG, H.-H. 2013. Economic costs of overweight and obesity. *Best Practice & Research Clinical Endocrinology & Metabolism,* 27**,** 105-115.

LEHR, S., HARTWIG, S. & SELL, H. 2012. Adipokines: a treasure trove for the discovery of biomarkers for metabolic disorders. *PROTEOMICS-Clinical Applications,* 6**,** 91-101.

LEJEUNE, M. P., KOVACS, E. M. & WESTERTERP-PLANTENGA, M. S. 2003. Effect of capsaicin on substrate oxidation and weight maintenance after modest body-weight loss in human subjects. *The British journal of nutrition,* 90**,** 651-59.

LEONHARDT, M. & LANGHANS, W. 2004. Fatty acid oxidation and control of food intake. *Physiology & Behavior,* 83**,** 645-651.

LEUNG, F. W. 2008. Capsaicin-sensitive intestinal mucosal afferent mechanism and body fat distribution. *Life sciences,* 83**,** 1-5.

LEUNG, F. W., GO, V. L. W., SCREMIN, O. U., OBENAUS, A., TUCK, M. L., GOLUB, M. S., EGGENA, P. & LEUNG, J. W. 2007. Pilot studies to demonstrate that intestinal mucosal afferent nerves are functionally linked to visceral adipose tissue. *Digestive diseases and sciences,* 52**,** 2695-2702.

LI, X., JIANG, Y., HU, N., LI, Y., ZHANG, M., HUANG, Z. & ZHAO, W. 2012. Prevalence and characteristic of overweight and obesity among adults in China, 2010. *Chinese journal of preventive medicine,* 46**,** 683-686.

LIM, K., YOSHIOKA, M., KIKUZATO, S., KIYONAGA, A., TANAKA, H., SHINDO, M. & SUZUKI, M. 1997. Dietary red pepper ingestion increases carbohydrate oxidation at rest and during exercise in runners. *Medicine and science in sports and exercise,* 29**,** 355-61.

LISHKO, P. V., PROCKO, E., JIN, X., PHELPS, C. B. & GAUDET, R. 2007. The ankyrin repeats of TRPV1 bind multiple ligands and modulate channel sensitivity. *Neuron,* 54**,** 905-918.

LIU, M., HUANG, W., WU, D. & PRIESTLEY, J. V. 2006. TRPV1, but not P2X3, requires cholesterol for its function and membrane expression in rat nociceptors. *European Journal of Neuroscience,* 24**,** 1-6.

LIX, L., KESELMAN, J. & KESELMAN, H. 1996. Consequences of assumption violations revisited: A quantitative review of alternatives to the one-way analysis of variance F test. *Review of educational research,* 66**,** 579-619.

LOOK AHEAD RESEARCH GROUP 2003. Look AHEAD (Action for Health in Diabetes): design and methods for a clinical trial of weight loss for the prevention of cardiovascular disease in type 2 diabetes. *Controlled clinical trials,* 24**,** 610-628.

LOOK AHEAD RESEARCH GROUP 2010. Long-term effects of a lifestyle intervention on weight and cardiovascular risk factors in individuals with type 2 diabetes mellitus: four-year results of the Look AHEAD trial. *Archives of internal medicine,* 170**,** 1566.

LUDY, M. & MATTES, R. 2011a. The effects of hedonically acceptable red pepper doses on thermogenesis and appetite. *Physiology & Behavior,* 102**,** 251-258.

LUDY, M. & MATTES, R. 2011b. The effects of hedonically acceptable red pepper doses on thermogenesis and appetite. *Physiology & behavior,* 102**,** 251-258.

LUDY, M., MOORE, G. & MATTES, R. 2012. The Effects of Capsaicin and Capsiate on Energy Balance: Critical Review and Meta-analyses of Studies in Humans. *Chemical Senses,* 37**,** 103-121.

LUO, X. J., PENG, J. & LI, Y. J. 2011. Recent advances in the study on capsaicinoids and capsinoids. *European journal of pharmacology,* 650**,** 1-7.

LÓPEZ-CARRILLO, L., LÓPEZ-CERVANTES, M., ROBLES-DÍAZ, G., RAMÍREZ-ESPITIA, A., MOHAR-BETANCOURT, A., MENESES-GARCÍA, A., LÓPEZ-VIDAL, Y. & BLAIR, A. 2003. Capsaicin consumption, Helicobacter pylori positivity and gastric cancer in Mexico. *International journal of cancer,* 106**,** 277-282.

MACLEAN, P. S., BERGOUIGNAN, A., CORNIER, M.-A. & JACKMAN, M. R. 2011. Biology's response to dieting: the impetus for weight regain. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology,* 301**,** R581-R600.

MAJOR, G., DOUCET, E., TRAYHURN, P., ASTRUP, A. & TREMBLAY, A. 2007. Clinical significance of adaptive thermogenesis. *International journal of obesity,* 31**,** 204-212.

MALIK, V. S., WILLETT, W. C. & HU, F. B. 2013. Global obesity: trends, risk factors and policy implications. *Nature Reviews Endocrinology,* 9**,** 13-27.

MANDADI, S. & ROUFOGALIS, B. D. 2008. ThermoTRP channels in nociceptors: taking a lead from capsaicin receptor TRPV1. *Current neuropharmacology,* 6**,** 21.

MANJUNATHA, H. & SRINIVASAN, K. 2007. Hypolipidemic and antioxidant effects of curcumin and capsaicin in high-fat-fed rats. *Canadian journal of physiology and pharmacology,* 85**,** 588-596.

MANN, T., TOMIYAMA, J., WESTLING, E., LEW, A., SAMUELS, B. & CHATMAN, J. 2007. Medicare's search for effective obesity treatments: diets are not the answer. *American Psychologist,* 62**,** 220.

MATTES, R. D. 2012. Spices and energy balance. *Physiology & Behavior,* 107**,** 584-590.

MAUGHAN, R. J., SHIRREFFS, S. M. & LEIPER, J. B. 2007. Errors in the estimation of hydration status from changes in body mass. *Journal of sports sciences,* 25**,** 797-804.

MCGAVIGAN, A. K. & MURPHY, K. G. 2012. Gut hormones: the future of obesity treatment? *British Journal of Clinical Pharmacology,* 74**,** 911-919.

MCGEE, H. 2004. *On Food And Cooking: The Science and Lore of the Kitchen*, Scribner.

MEGHVANSI, M., SIDDIQUI, S., KHAN, M., GUPTA, V., VAIRALE, M., GOGOI, H. & SINGH, L. 2010. Naga chilli: A potential source of capsaicinoids with broad-spectrum ethnopharmacological applications. *Journal of Ethnopharmacology,* 132**,** 1-14.

MEI, Z., GRUMMER-STRAWN, L. M., PIETROBELLI, A., GOULDING, A., GORAN, M. I. & DIETZ, W. H. 2002. Validity of body mass index compared with other body-composition screening indexes for the assessment of body fatness in children and adolescents. *The American journal of clinical nutrition,* 75**,** 978-985.

MENIGOZ, A. & BOUDES, M. 2011. The expression pattern of TRPV1 in brain. *The Journal of Neuroscience,* 31**,** 13025-13027.

MILLER, L. E. 2013. Lorcaserin for weight loss: insights into US Food and Drug Administration approval. *J. Acad. Nutr. Diet,* 113**,** 25-30.

MOYER, V. A. 2012. Screening for and management of obesity in adults: US Preventive Services Task Force recommendation statement. *Annals of internal medicine,* 157**,** 373-378.

MUENNIG, P., LUBETKIN, E., JIA, H. & FRANKS, P. 2006. Gender and the burden of disease attributable to obesity. *American Journal of Public Health,* 96**,** 1662-1668.

MYERS, J., WELL, A. & LORCH, R. 2010. *Research design and statistical analysis*, Routledge.

MÓZSIK, G., SZOLCSÁNYI, J. & DÖMÖTÖR, A. 2007. Capsaicin research as a new tool to approach of the human gastrointestinal physiology, pathology and pharmacology. *Inflammopharmacology,* 15**,** 232-245.

NAKAMURA, K. & MORRISON, S. 2007. Central efferent pathways mediating skin cooling-evoked sympathetic thermogenesis in brown adipose tissue. *American*

Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 292**,** R127-R136.

NEDERGAARD, J., BENGTSSON, T. & CANNON, B. 2007. Unexpected evidence for active brown adipose tissue in adult humans. *American Journal of Physiology-Endocrinology And Metabolism,* 293**,** E444-E452.

NELSON, E. 1919. The Constitution of Capsaicin, the Pungent Principle of Capsicum. *Journal of the American Chemical Society,* 41**,** 1115-1121.

NEUHOUSER, M. L., TINKER, L., SHAW, P. A., SCHOELLER, D., BINGHAM, S. A., VAN HORN, L., BERESFORD, S. A., CAAN, B., THOMSON, C. & SATTERFIELD, S. 2008. Use of recovery biomarkers to calibrate nutrient consumption self-reports in the Women's Health Initiative. *American journal of epidemiology,* 167**,** 1247-1259.

NGUYEN, A., HERZOG, H. & SAINSBURY, A. 2011a. Neuropeptide Y and peptide YY: important regulators of energy metabolism. *Current Opinion in Endocrinology, Diabetes and Obesity,* 18**,** 56-60.

NGUYEN, N., NGUYEN, X., LANE, J. & WANG, P. 2011b. Relationship between obesity and diabetes in a US adult population: findings from the National Health and Nutrition Examination Survey, 1999–2006. *Obesity surgery,* 21**,** 351-355.

NHLBI 2011. *The practical guide: identification, evaluation, and treatment of overweight and obesity in adults*, National Heart, Lung, and Blood Institute.

NICHOLS, M., DE SILVA-SANIGORSKI, A., CLEARY, J., GOLDFELD, S., COLAHAN, A. & SWINBURN, B. 2011. Decreasing trends in overweight and obesity among an Australian population of preschool children. *International journal of obesity,* 35**,** 916-924.

NILIUS, B. & APPENDINO, G. 2011. Tasty and healthy TR (i) Ps. *EMBO reports,* 12**,** 1094-1101.

OCHI, T., TAKAISHI, Y., KOGURE, K. & YAMAUTI, I. 2003. Antioxidant Activity of a New Capsaicin Derivative from Capsicum a nnuum. *Journal of natural products,* 66**,** 1094-1096.

OGDEN, C. 2012. Prevalence of obesity in the United States, 2009-2010. US Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Health Statistics.

OH, S. H., KIM, Y. S., LIM, S. C., HOU, Y. F., CHANG, I. Y. & YOU, H. J. 2008. Dihydrocapsaicin (DHC), a saturated structural analog of capsaicin, induces autophagy in human cancer cells in a catalase-regulated manner. *Autophagy,* 4**,** 1009-1019.

OLSHANSKY, S. J., PASSARO, D. J., HERSHOW, R. C., LAYDEN, J., CARNES, B. A., BRODY, J., HAYFLICK, L., BUTLER, R. N., ALLISON, D. B. & LUDWIG, D.

S. 2005. A potential decline in life expectancy in the United States in the 21st century. *New England Journal of Medicine,* 352**,** 1138-1145.

OSMOND, J. M., MINTZ, J. D., DALTON, B. & STEPP, D. W. 2009. Obesity increases blood pressure, cerebral vascular remodeling, and severity of stroke in the Zucker rat. *Hypertension,* 53**,** 381-386.

OUCHI, N., PARKER, J. L., LUGUS, J. J. & WALSH, K. 2011. Adipokines in inflammation and metabolic disease. *Nature Reviews Immunology,* 11**,** 85-97.

O'NEIL, R. G. & BROWN, R. C. 2003. The vanilloid receptor family of calcium-permeable channels: molecular integrators of microenvironmental stimuli. *Physiology,* 18**,** 226-231.

PADWAL, R., KLARENBACH, S., WIEBE, N., BIRCH, D., KARMALI, S., MANNS, B., HAZEL, M., SHARMA, A. & TONELLI, M. 2011. Bariatric surgery: a systematic review and network meta‐analysis of randomized trials. *Obesity reviews,* 12**,** 602-621.

PALOMO, I., ALARCÓN, M., MOORE-CARRASCO, R. & ARGILÉS, J. M. 2006. Hemostasis alterations in metabolic syndrome (Review). *International journal of molecular medicine,* 18**,** 969.

PANOULAS, V., AHMAD, N., FAZAL, A., KASSAMALI, R., NIGHTINGALE, P., KITAS, G. & LABIB, M. 2008. The inter-operator variability in measuring waist circumference and its potential impact on the diagnosis of the metabolic syndrome. *Postgraduate medical journal,* 84**,** 344-347.

PEETERS, A., BARENDREGT, J. J., WILLEKENS, F., MACKENBACH, J. P., AL MAMUN, A. & BONNEUX, L. 2003. Obesity in adulthood and its consequences for life expectancy: a life-table analysis. *Annals of internal medicine,* 138**,** 24-32.

PELLOWE, E., SALAKO, K., SNELLING, S., URDAIBAY, C. & WESTON, C. 2010. Daily variability in waist circumference. *Heart-London,* 96**,** 550.

PELONE, F., SPECCHIA, M. L., VENEZIANO, M. A., CAPIZZI, S., BUCCI, S., MANCUSO, A., RICCIARDI, W. & DE BELVIS, A. 2012. Economic impact of childhood obesity on health systems: a systematic review. *Obesity reviews,* 13**,** 431-440.

PENG, J. & LI, Y.-J. 2010. The vanilloid receptor TRPV1: role in cardiovascular and gastrointestinal protection. *European journal of pharmacology,* 627**,** 1-7.

PERRY, L., DICKAU, R., ZARRILLO, S., HOLST, I., PEARSALL, D. M., PIPERNO, D. R., BERMAN, M. J., COOKE, R. G., RADEMAKER, K. & RANERE, A. J. 2007. Starch fossils and the domestication and dispersal of chili peppers (Capsicum spp. L.) in the Americas. *Science,* 315**,** 986-988.

PHELAN, S., KANAYA, A. M., SUBAK, L. L., HOGAN, P. E., ESPELAND, M. A., WING, R. R., BURGIO, K. L., DILILLO, V., GORIN, A. A. & WEST, D. S. 2012. Weight loss prevents urinary incontinence in women with type 2 diabetes: results from the Look AHEAD trial. *The Journal of urology,* 187**,** 939-944.

PHUNG, O. J., BAKER, W. L., MATTHEWS, L. J., LANOSA, M., THORNE, A. & COLEMAN, C. I. 2010. Effect of green tea catechins with or without caffeine on anthropometric measures: a systematic review and meta-analysis. *The American journal of clinical nutrition,* 91**,** 73-81.

PIMENTEL, G., BERNHARD, A., FREZZA, M., RINALDI, A. & BURINI, R. 2010. Bioelectric impedance overestimates the body fat in overweight and underestimates in Brazilian obese women: a comparation with Segal equation 1. *Nutrición Hospitalaria,* 25**,** 741.

PINKNEY, J. 2011. The surgical panacea for diabetes: time for diabetologists to discriminate facts from fiction and flights of fantasy. *Practical Diabetes International,* 28**,** 76-80.

POCOCK, G., RICHARDS, C. D. & RICHARDS, D. A. 2013. *Human physiology*, Oxford university press.

POPKIN, B. M., ADAIR, L. S. & NG, S. W. 2012. Global nutrition transition and the pandemic of obesity in developing countries. *Nutrition reviews,* 70**,** 3-21.

POPKIN, B. M. & GORDON-LARSEN, P. 2004. The nutrition transition: worldwide obesity dynamics and their determinants. *International journal of obesity,* 28**,** S2-S9.

PORSZASZ, J. & JANCSO, N. 1959. Studies on the action potentials of sensory nerves in animals desensitized with capsaicine. *Acta physiologica Academiae Scientiarum Hungaricae,* 16**,** 299.

PRAKASH, U. N. & SRINIVASAN, K. 2010. Beneficial influence of dietary spices on the ultrastructure and fluidity of the intestinal brush border in rats. *British Journal of Nutrition,* 104**,** 31-39.

PRAKASH, U. N. & SRINIVASAN, K. 2013. Enhanced intestinal uptake of iron, zinc and calcium in rats fed pungent spice principles–Piperine, capsaicin and ginger (< i> Zingiber officinale</i>). *Journal of Trace Elements in Medicine and Biology,* 27**,** 184-190.

PRENTICE, A. M. & JEBB, S. A. 2001. Beyond body mass index. *Obesity reviews,* 2**,** 141-147.

RABKIN, S., CHEN, Y., LEITER, L., LIU, L. & REEDER, B. 1997. Risk factor correlates of body mass index. Canadian Heart Health Surveys Research Group. *CMAJ: Canadian Medical Association journal= journal de l'Association medicale canadienne,* 157**,** S26.

RAGHAVENDRA, R. & NAIDU, K. A. 2009. Spice active principles as the inhibitors of human platelet aggregation and thromboxane biosynthesis. *Prostaglandins, Leukotrienes and Essential Fatty Acids,* 81**,** 73-78.

RAMACHANDRA, R. & RAVISHANKAR, G. 2002. Plant cell cultures: chemical factories of secondary metabolites. *Biotechnology advances,* 20**,** 101-153.

RAMSEY, I. S., DELLING, M. & CLAPHAM, D. E. 2006. An introduction to TRP channels. *Annu. Rev. Physiol.,* 68**,** 619-647.

RANKINEN, T., KIM, S., PERUSSE, L., DESPRES, J. & BOUCHARD, C. 1999. The prediction of abdominal visceral fat level from body composition and anthropometry: ROC analysis. *International journal of obesity and related metabolic disorders: journal of the International Association for the Study of Obesity,* 23**,** 801-809.

RAPPANGE, D. R., VAN BAAL, P. H., VAN EXEL, N. J. A., FEENSTRA, T. L., RUTTEN, F. F. & BROUWER, W. B. 2008. Unrelated medical costs in Life-Years gained. *Pharmacoeconomics,* 26**,** 815-830.

RAYBOULD, H. E. 2013. The heat is on: does direct application of capsaicin to autonomic nerves produce a specific deafferentation? *The Journal of physiology,* 591**,** 1405-1405.

REHMAN, R., BHAT, Y., PANDA, L. & MABALIRAJAN, U. 2013. TRPV1 inhibition attenuates IL-13 mediated asthma features in mice by reducing airway epithelial injury. *International immunopharmacology,* 15**,** 597-605.

REILLY, C., CROUCH, D. & YOST, G. 2001. Quantitative analysis of capsaicinoids in fresh peppers, oleoresin capsicum, and pepper spray products. *Journal of forensic sciences,* 46**,** 502-509.

REILLY, C., HENION, F., BUGNI, T., ETHIRAJAN, M., STOCKMANN, C., PRAMANIK, K., SRIVASTAVA, S. & YOST, G. 2012. Reactive Intermediates Produced from the Metabolism of the Vanilloid Ring of Capsaicinoids by P450 Enzymes. *Chemical Research in Toxicology,* 26**,** 55-66.

REINBACH, H. C., MARTINUSSEN, T. & MOLLER, P. 2010. Effects of hot spices on energy intake, appetite and sensory specific desires in humans. *Food Quality and Preference,* 21**,** 655-661.

REINBACH, H. C., SMEETS, A., MARTINUSSEN, T., MOLLER, P. & WESTERTERP-PLANTENGA, M. S. 2009. Effects of capsaicin, green tea and CH-19 sweet pepper on appetite and energy intake in humans in negative and positive energy balance. *Clinical nutrition,* 28**,** 260-5.

REJESKI, W. J., IP, E. H., BERTONI, A. G., BRAY, G. A., EVANS, G., GREGG, E. W. & ZHANG, Q. 2012. Lifestyle change and mobility in obese adults with type 2 diabetes. *New England Journal of Medicine,* 366**,** 1209-1217.

REXRODE, K. M., HENNEKENS, C. H., WILLETT, W. C., COLDITZ, G. A., STAMPFER, M. J., RICH-EDWARDS, J. W., SPEIZER, F. E. & MANSON, J. E. 1997. A prospective study of body mass index, weight change, and risk of stroke in women. *JAMA: the journal of the American Medical Association,* 277**,** 1539-1545.

REYES-ESCOGIDO, M. D. L., GONZALEZ-MONDRAGON, E. G. & VAZQUEZ-TZOMPANTZI, E. 2011. Chemical and pharmacological aspects of capsaicin. *Molecules,* 16**,** 1253-1270.

RIMM, E. B., STAMPFER, M. J., GIOVANNUCCI, E., ASCHERIO, A., SPIEGELMAN, D., COLDITZ, G. A. & WILLETT, W. C. 1995. Body size and fat distribution as predictors of coronary heart disease among middle-aged and older US men. *American Journal of Epidemiology,* 141**,** 1117-1127.

ROBERTS, S. B. 2000. Energy regulation and aging: recent findings and their implications. *Nutrition reviews,* 58**,** 91-97.

ROBERTS, S. B. & ROSENBERG, I. 2006. Nutrition and aging: changes in the regulation of energy metabolism with aging. *Physiological reviews,* 86**,** 651-667.

RODGERS, R. J., TSCHÖP, M. H. & WILDING, J. P. 2012. Anti-obesity drugs: past, present and future. *Disease models & mechanisms,* 5**,** 621-626.

ROKHOLM, B., BAKER, J. & SØRENSEN, T. 2010. The levelling off of the obesity epidemic since the year 1999–a review of evidence and perspectives. *Obesity reviews,* 11**,** 835-846.

ROLLS, E. 2004a. Smell, taste, texture, and temperature multimodal representations in the brain, and their relevance to the control of appetite. *Nutrition reviews,* 62**,** 193-204.

ROLLS, E. 2004b. The functions of the orbitofrontal cortex. *Brain and cognition,* 55**,** 11-29.

ROLLS, E. & GRABENHORST, F. 2008. The orbitofrontal cortex and beyond: from affect to decision-making. *Progress in neurobiology,* 86**,** 216-44.

ROSENBAUM, M., HIRSCH, J., GALLAGHER, D. A. & LEIBEL, R. L. 2008. Long-term persistence of adaptive thermogenesis in subjects who have maintained a reduced body weight. *The American journal of clinical nutrition,* 88**,** 906-912.

ROTH, J., QIANG, X., MARBÁN, S. L., REDELT, H. & LOWELL, B. C. 2004. The obesity pandemic: where have we been and where are we going? *Obesity Research,* 12**,** 88S-101S.

RUBIN, R., GAUSSOIN, S., PEYROT, M., DILILLO, V., MILLER, K., WADDEN, T., WEST, D., WING, R. & KNOWLER, W. 2010. Cardiovascular disease risk factors, depression symptoms and antidepressant medicine use in the Look

AHEAD (Action for Health in Diabetes) clinical trial of weight loss in diabetes. *Diabetologia,* 53**,** 1581-1589.

SACKS, F. M., BRAY, G. A., CAREY, V. J., SMITH, S. R., RYAN, D. H., ANTON, S. D., MCMANUS, K., CHAMPAGNE, C. M., BISHOP, L. M. & LARANJO, N. 2009. Comparison of weight-loss diets with different compositions of fat, protein, and carbohydrates. *New England Journal of Medicine,* 360**,** 859-873.

SAINSBURY, A. & ZHANG, L. 2010. Role of the arcuate nucleus of the hypothalamus in regulation of body weight during energy deficit. *Molecular and cellular endocrinology,* 316**,** 109-119.

SAITO, A. & YAMAMOTO, M. 1996. Acute oral toxicity of capsaicin in mice and rats. *The Journal of toxicological sciences,* 21**,** 195-200.

SAITO, M., OKAMATSU-OGURA, Y., MATSUSHITA, M., WATANABE, K., YONESHIRO, T., NIO-KOBAYASHI, J., IWANAGA, T., MIYAGAWA, M., KAMEYA, T. & NAKADA, K. 2009. High incidence of metabolically active brown adipose tissue in healthy adult humans effects of cold exposure and adiposity. *Diabetes,* 58**,** 1526-1531.

SAITO, M. & YONESHIRO, T. 2013. Capsinoids and related food ingredients activating brown fat thermogenesis and reducing body fat in humans. *Current opinion in lipidology,* 24**,** 71-77.

SAMBAIAH, K. & SATYANARAYANA, M. 1982. Influence of red pepper and capsaicin on body composition and lipogenesis in rats. *Journal of Biosciences,* 4**,** 425-430.

SASSI, F. 2010. *Obesity and the economics of prevention: fit not fat*, OECD Publishing.

SATHANANTHAN, M., FARRUGIA, L. P., MILES, J. M., PICCININI, F., DALLA MAN, C., ZINSMEISTER, A. R., COBELLI, C., RIZZA, R. A. & VELLA, A. 2013. Direct effects of exendin-(9, 39) and GLP-1-(9, 36) amide on insulin action, β-cell function, and glucose metabolism in nondiabetic subjects. *Diabetes,* 62**,** 2752-2756.

SAWYNOK, J. 2005. Topical analgesics in neuropathic pain. *Current pharmaceutical design,* 11**,** 2995-3004.

SCHMITZ, J., SAYRE, S., STOTTS, A., ROTHFLEISCH, J. & MOONEY, M. 2005. Medication compliance during a smoking cessation clinical trial: a brief intervention using MEMS feedback. *Journal of behavioral medicine,* 28**,** 139-147.

SCHUTZ, Y., TREMBLAY, A., WEINSIER, R. L. & NELSON, K. M. 1992. Role of fat oxidation in the long-term stabilization of body weight in obese women. *The American journal of clinical nutrition,* 55**,** 670-674.

SCHWARTZ, M. W., WOODS, S. C., PORTE, D., SEELEY, R. J. & BASKIN, D. G. 2000. Central nervous system control of food intake. *Nature,* 404**,** 661-671.

SCOVILLE, W. L. 1912. Note on capsicums. *Journal of the American Pharmaceutical Association,* 1**,** 453-454.

SEALE, P., KAJIMURA, S. & SPIEGELMAN, B. M. 2009. Transcriptional control of brown adipocyte development and physiological function—of mice and men. *Genes & development,* 23**,** 788-797.

SEALE, P. & LAZAR, M. A. 2009. Brown fat in humans: turning up the heat on obesity. *Diabetes,* 58**,** 1482-1484.

SEIDELL, J. C. 2014. 4 Worldwide Prevalence of Obesity in Adults. *Handbook of Obesity: Epidemology, Etiology, and Physiopathology,* 1**,** 47.

SEN, B., MAHALANABIS, D., KURPAD, A. V., SHAIKH, S. & BOSE, K. 2010. Total body water and fat-free mass: evaluation of equations based on bioelectrical impedance analysis in infants and young children in India. *British Journal of Nutrition,* 104**,** 256-264.

SHARMA, S. K., VIJ, A. S. & SHARMA, M. 2013. Mechanisms and clinical uses of capsaicin. *European journal of pharmacology,* 720**,** 55-62.

SHEN, C.-L., YEH, J., CAO, J., TATUM, O., DAGDA, R. & WANG, J.-S. 2010. Synergistic effects of green tea polyphenols and alphacalcidol on chronic inflammation-induced bone loss in female rats. *Osteoporosis international,* 21**,** 1841-1852.

SHIN, K. & MORITANI, T. 2007. The combined effects of capsaicin, green tea extract and chicken essence tablets on human autonomic nervous system activity. *Journal of nutritional science and vitaminology,* 53**,** 145-52.

SHUBHA, M. C., REDDY, R. R. & SRINIVASAN, K. 2011. Antilithogenic influence of dietary capsaicin and curcumin during experimental induction of cholesterol gallstone in mice. *Applied Physiology, Nutrition, and Metabolism,* 36**,** 201-209.

SINGH, A., KIRAN, D., SINGH, H., NEL, B., SINGH, P. & TIWARI, P. 2008. Prevalence and severity of dysmenorrhea: A problem related to menstruation, among first and second year female medical students.

SJÖSTRÖM, L., RISSANEN, A., ANDERSEN, T., BOLDRIN, M., GOLAY, A., KOPPESCHAAR, H. P. & KREMPF, M. 1998. Randomised placebo-controlled trial of orlistat for weight loss and prevention of weight regain in obese patients. *The Lancet,* 352**,** 167-172.

SMEETS, A., JANSSENS, P. & WESTERTERP-PLANTENGA, M. 2013. Addition of capsaicin and exchange of carbohydrate with protein counteract energy intake restriction effects on fullness and energy expenditure. *The Journal of nutrition,* 143**,** 442-447.

SMEETS, A. & WESTERTERP-PLANTENGA, M. 2009. The acute effects of a lunch containing capsaicin on energy and substrate utilisation, hormones, and satiety. *European Journal of Nutrition,* 48**,** 229-234.

SMITH, R. & THORNER, M. 2012. *Ghrelin in Health and Disease*, Springer.

SMITH, S., WEISSMAN, N., ANDERSON, C., SANCHEZ, M., CHUANG, E., STUBBE, S., BAYS, H. & SHANAHAN, W. 2010. Multicenter, placebo-controlled trial of lorcaserin for weight management. *New England Journal of Medicine,* 363**,** 245-256.

SNITKER, S., FUJISHIMA, Y., SHEN, H., OTT, S., PI-SUNYER, X., FURUHATA, Y., SATO, H. & TAKAHASHI, M. 2009. Effects of novel capsinoid treatment on fatness and energy metabolism in humans: possible pharmacogenetic implications. *The American journal of clinical nutrition,* 89**,** 45-50.

SRINIVASAN, M. R. & CHANDRASEKHARA, N. 1992. Comparative influence of vanillin & capsaicin on liver & blood lipids in the rat. *Indian J Med Res,* 96**,** 133-5.

STEARNS, A., BALAKRISHNAN, A., RADMANESH, A., ASHLEY, S., RHOADS, D. & TAVAKKOLIZADEH, A. 2012. Relative contributions of afferent vagal fibers to resistance to diet-induced obesity. *Digestive diseases and sciences,* 57**,** 1281-1290.

STERN, L., IQBAL, N., SESHADRI, P., CHICANO, K. L., DAILY, D. A., MCGRORY, J., WILLIAMS, M., GRACELY, E. J. & SAMAHA, F. F. 2004. The effects of low-carbohydrate versus conventional weight loss diets in severely obese adults: one-year follow-up of a randomized trial. *Annals of internal medicine,* 140**,** 778-85.

STOTHARD, K. J., TENNANT, P. W., BELL, R. & RANKIN, J. 2009. Maternal overweight and obesity and the risk of congenital anomalies: a systematic review and meta-analysis. *JAMA : the journal of the American Medical Association,* 301**,** 636-650.

STROHACKER, K., MCCAFFERY, J., MACLEAN, P. & WING, R. 2013. Adaptations of leptin, ghrelin or insulin during weight loss as predictors of weight regain: a review of current literature. *International journal of obesity,* 38**,** 388-396.

STUNKARD, A. & MESSICK, S. 1985. The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. *Journal of psychosomatic research,* 29**,** 71-83.

SUMITHRAN, P., PRENDERGAST, L. A., DELBRIDGE, E., PURCELL, K., SHULKES, A., KRIKETOS, A. & PROIETTO, J. 2011. Long-term persistence of hormonal adaptations to weight loss. *New England Journal of Medicine,* 365**,** 1597-1604.

SUN, G., FRENCH, C. R., MARTIN, G. R., YOUNGHUSBAND, B., GREEN, R. C., XIE, Y.-G., MATHEWS, M., BARRON, J. R., FITZPATRICK, D. G. &

GULLIVER, W. 2005. Comparison of multifrequency bioelectrical impedance analysis with dual-energy X-ray absorptiometry for assessment of percentage body fat in a large, healthy population. *The American journal of clinical nutrition,* 81**,** 74-78.

SUN, K., KUSMINSKI, C. M. & SCHERER, P. E. 2011. Adipose tissue remodeling and obesity. *The Journal of clinical investigation,* 121**,** 2094-2101.

SURESH, D. & SRINIVASAN, K. 2007. Studies on the in vitro absorption of spice principles–Curcumin, capsaicin and piperine in rat intestines. *Food and chemical toxicology,* 45**,** 1437-1442.

SURESH, D. & SRINIVASAN, K. 2010. Tissue distribution & elimination of capsaicin, piperine & curcumin following oral intake in rats. *Indian Journal of Medical Research,* 131.

SUZUKI, K., SIMPSON, K. A., MINNION, J. S., SHILLITO, J. C. & BLOOM, S. R. 2010. The role of gut hormones and the hypothalamus in appetite regulation. *Endocr J,* 57**,** 359-372.

SWIFT, D. L., JOHANNSEN, N. M., LAVIE, C. J., EARNEST, C. P. & CHURCH, T. S. 2013. The Role of Exercise and Physical Activity in Weight Loss and Maintenance. *Progress in cardiovascular diseases*.

SWINBURN, B. A., SACKS, G., HALL, K. D., MCPHERSON, K., FINEGOOD, D. T., MOODIE, M. L. & GORTMAKER, S. L. 2011. The global obesity pandemic: shaped by global drivers and local environments. *The Lancet,* 378**,** 804-814.

SWORDS, J. 2014. UK government spending in times of austerity. *Environment and Planning A,* 46**,** 2-4.

SZALLASI, A. & BLUMBERG, P. M. 1999. Vanilloid (capsaicin) receptors and mechanisms. *Pharmacological reviews,* 51**,** 159-212.

SZALLASI, A., CORTRIGHT, D. N., BLUM, C. A. & EID, S. R. 2007. The vanilloid receptor TRPV1: 10 years from channel cloning to antagonist proof-of-concept. *Nature Reviews Drug Discovery,* 6**,** 357-372.

SZOLCSANYI, J. & BARTHO, L. 2001. Capsaicin-sensitive afferents and their role in gastroprotection: an update. *Journal of Physiology-Paris,* 95**,** 181-188.

TATE, D. F., JEFFERY, R. W., SHERWOOD, N. E. & WING, R. R. 2007. Long-term weight losses associated with prescription of higher physical activity goals. Are higher levels of physical activity protective against weight regain? *The American journal of clinical nutrition,* 85**,** 954-959.

TAYLOR, R. & MARCHAND, V. 2010. Re: Promoting optimal monitoring of child growth in Canada: Using the new World Health Organization growth charts - Executive Summary. Paediatr Child Health 2010;15(2):77-83. *Paediatrics & Child Health,* 15**,** 258 - 258.

TEWKSBURY, J., MANCHEGO, C., HAAK, D. C. & LEVEY, D. J. 2006. Where did the chili get its spice? Biogeography of capsaicinoid production in ancestral wild chili species. *Journal of Chemical Ecology,* 32**,** 547-564.

TEWKSBURY, J. & NABHAN, G. 2001. Seed dispersal. Directed deterrence by capsaicin in chilies. *Nature,* 412**,** 403-404.

THOENNISSEN, N., O'KELLY, J., LU, D., IWANSKI, G., LA, D., ABBASSI, S., LEITER, A., KARLAN, B., MEHTA, R. & KOEFFLER, H. 2010. Capsaicin causes cell-cycle arrest and apoptosis in ER-positive and-negative breast cancer cells by modulating the EGFR/HER-2 pathway. *Oncogene,* 29**,** 285-296.

TOH, C., LEE, T. & KIANG, A. 1955. The pharmacological actions of capsaicin and analogues. *British journal of pharmacology and chemotherapy,* 10**,** 175-182.

TRAYHURN, P. & BEATTIE, J. H. 2001. Physiological role of adipose tissue: white adipose tissue as an endocrine and secretory organ. *Proceedings of the Nutrition Society,* 60**,** 329-339.

TROGDON, J., FINKELSTEIN, E., HYLANDS, T., DELLEA, P. & KAMAL‐BAHL, S. 2008. Indirect costs of obesity: a review of the current literature. *Obesity reviews,* 9**,** 489-500.

TRUSWELL, A. S. 2010. *Cholesterol and Beyond,* Berlin, Springer.

UNICK, J. L., BEAVERS, D., JAKICIC, J. M., KITABCHI, A. E., KNOWLER, W. C., WADDEN, T. A. & WING, R. R. 2011. Effectiveness of Lifestyle Interventions for Individuals With Severe Obesity and Type 2 Diabetes Results from the Look AHEAD trial. *Diabetes Care,* 34**,** 2152-2157.

VAINIO, H., KAAKS, R. & BIANCHINI, F. 2002. Weight control and physical activity in cancer prevention: international evaluation of the evidence. *European journal of cancer prevention: the official journal of the European Cancer Prevention Organisation (ECP),* 11**,** S94.

VAN DER LELY, A. J., TSCHOP, M., HEIMAN, M. L. & GHIGO, E. 2004. Biological, physiological, pathophysiological, and pharmacological aspects of ghrelin. *Endocrine reviews,* 25**,** 426-457.

VAN GAAL, L. F., MERTENS, I. L. & CHRISTOPHE, E. 2006. Mechanisms linking obesity with cardiovascular disease. *Nature,* 444**,** 875-880.

VERVLOET, M., LINN, A., VAN WEERT, J., DE BAKKER, D., BOUVY, M. & VAN DIJK, L. 2012. The effectiveness of interventions using electronic reminders to improve adherence to chronic medication: a systematic review of the literature. *Journal of the American Medical Informatics Association,* 19**,** 696-704.

VEST, A. R., HENEGHAN, H. M., SCHAUER, P. R. & YOUNG, J. B. 2013. Surgical management of obesity and the relationship to cardiovascular disease. *Circulation,* 127**,** 945-959.

VIRTUE, S. & VIDAL-PUIG, A. 2010. Adipose tissue expandability, lipotoxicity and the metabolic syndrome—an allostatic perspective. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids,* 1801**,** 338-349.

VISSCHER, T. & SEIDELL, J. 2004. Time trends (1993–1997) and seasonal variation in body mass index and waist circumference in the Netherlands. *International journal of obesity,* 28**,** 1309-1316.

VIVANTI, A., YU, L., PALMER, M., DAKIN, L., SUN, J. & CAMPBELL, K. 2013. Short‐term body weight fluctuations in older well‐hydrated hospitalised patients. *Journal of Human Nutrition and Dietetics,* 26**,** 429-435.

WADDEN, T. A., BUTRYN, M. L. & WILSON, C. 2007. Lifestyle modification for the management of obesity. *Gastroenterology,* 132**,** 2226-2238.

WADDEN, T. A. & FOSTER, G. D. 2000. Behavioral treatment of obesity. *Medical Clinics of North America,* 84**,** 441-461.

WADDEN, T. A., VOLGER, S., TSAI, A. G., SARWER, D. B., BERKOWITZ, R. I., DIEWALD, L., CARVAJAL, R., MORAN, C. H. & VETTER, M. 2013. Managing obesity in primary care practice: an overview with perspective from the POWER-UP study. *International journal of obesity,* 37**,** S3-S11.

WADDEN, T. A., WEBB, V. L., MORAN, C. H. & BAILER, B. A. 2012. Lifestyle modification for obesity new developments in diet, physical activity, and behavior therapy. *Circulation,* 125**,** 1157-1170.

WADDEN, T. A., WEST, D. S., NEIBERG, R. H., WING, R. R., RYAN, D. H., JOHNSON, K. C., FOREYT, J. P., HILL, J. O., TRENCE, D. L. & VITOLINS, M. Z. 2009. One‐year Weight Losses in the Look AHEAD Study: Factors Associated With Success. *Obesity,* 17**,** 713-722.

WAKIL, S. 2012. *Lipid metabolism,* Amsterdam, Elsevier.

WALSH, B. M. & HOOT, S. B. 2001. Phylogenetic relationships of Capsicum (Solanaceae) using DNA sequences from two noncoding regions: the chloroplast atpB‐rbcL spacer region and nuclear waxy introns. *International Journal of Plant Sciences,* 162**,** 1409-1418.

WANG, L., HU, C., DENG, P., SHEN, S., ZHU, H., DING, J., TAN, G. & LI, Y. 2005. The protective effects of rutaecarpine on gastric mucosa injury in rats. *Planta medica,* 71**,** 416-419.

WANG, P., YAN, Z., ZHONG, J., CHEN, J., NI, Y., LI, L., MA, L., ZHAO, Z., LIU, D. & ZHU, Z. 2012. Transient receptor potential vanilloid 1 activation enhances gut glucagon-like peptide-1 secretion and improves glucose homeostasis. *Diabetes,* 61**,** 2155-65.

WANG, Y., BEYDOUN, M. A., LIANG, L., CABALLERO, B. & KUMANYIKA, S. K. 2008. Will all Americans become overweight or obese? Estimating the progression and cost of the US obesity epidemic. *Obesity,* 16**,** 2323-2330.

WANG, Y., MCPHERSON, K., MARSH, T., GORTMAKER, S. & BROWN, M. 2011. Health and economic burden of the projected obesity trends in the USA and the UK. *The Lancet,* 378**,** 815-825.

WATANABE, T., KAWADA, T., YAMAMOTO, M. & IWAI, K. 1987. Capsaicin, a pungent principle of hot red pepper, evokes catecholamine secretion from the adrenal medulla of anesthetized rats. *Biochemical and biophysical research communications,* 142**,** 259-64.

WEERAPAN, K. 2009. Pharmacokinetic and the effect of capsaicin in Capsicum frutescens on decreasing plasma glucose level. *J Med Assoc Thai,* 92**,** 108-13.

WEISSMAN, N. J. 2001. Appetite suppressants and valvular heart disease. *The American journal of the medical sciences,* 321**,** 285-291.

WELLMAN, P. J. 2000. Norepinephrine and the control of food intake. *Nutrition,* 16**,** 837-842.

WESTERTERP-PLANTENGA, M., SMEETS, A. & LEJEUNE, M. 2005. Sensory and gastrointestinal satiety effects of capsaicin on food intake. *International journal of obesity,* 29**,** 682-8.

WHITING, S., DERBYSHIRE, E. & TIWARI, B. 2012. Capsaicinoids and capsinoids. A potential role for weight management? A systematic review of the evidence. *Appetite,* 59**,** 341-348.

WHITING, S., DERBYSHIRE, E. & TIWARI, B. 2014. Could capsaicinoids help to support weight management? A systematic review and meta-analysis of energy intake data. *Appetite,* 73**,** 183-188.

WHO 2008. Waist Circumference and Waist-Hip Ratio, Report of a WHO Expert Consultation. *In:* WHO (ed.).

WHO 2011. *World health statistics 2011,* Geneva, Switzerland, World Health Organization.

WILLETT, W. 2013. *Nutritional epidemiology,* Oxford, Oxford University Press.

WILLIAMS, G. 2010. Withdrawal of sibutramine in Europe. *BMJ,* 340.

WILSON, P. W., D'AGOSTINO, R. B., SULLIVAN, L., PARISE, H. & KANNEL, W. B. 2002. Overweight and obesity as determinants of cardiovascular risk: the Framingham experience. *Archives of internal medicine,* 162**,** 1867-1872.

WING, R. R., LANG, W., WADDEN, T. A., SAFFORD, M., KNOWLER, W. C., BERTONI, A. G., HILL, J. O., BRANCATI, F. L., PETERS, A. & WAGENKNECHT, L. 2011. Benefits of modest weight loss in improving

cardiovascular risk factors in overweight and obese individuals with type 2 diabetes. *Diabetes Care,* 34**,** 1481-1486.

WING, R. R. & PHELAN, S. 2005. Long-term weight loss maintenance. *The American journal of clinical nutrition,* 82**,** 222S-225S.

WITHROW, D. & ALTER, D. 2011. The economic burden of obesity worldwide: a systematic review of the direct costs of obesity. *Obesity reviews,* 12**,** 131-141.

WITTEMAN, J., WILLETT, W. C., STAMPFER, M. J., COLDITZ, G. A., SACKS, F. M., SPEIZER, F. E., ROSNER, B. & HENNEKENS, C. H. 1989. A prospective study of nutritional factors and hypertension among US women. *Circulation,* 80**,** 1320-1327.

WOLFE, S. M. 2013. When EMA and FDA decisions conflict: differences in patients or in regulation? *BMJ: British Medical Journal,* 347.

WOLFENSTETTER, S. 2012. Future direct and indirect costs of obesity and the influence of gaining weight: results from the MONICA/KORA cohort studies, 1995–2005. *Economics & Human Biology,* 10**,** 127-138.

WOLIN, K. Y., CARSON, K. & COLDITZ, G. A. 2010. Obesity and cancer. *The oncologist,* 15**,** 556-565.

WOLOSHIN, S. & SCHWARTZ, L. M. 2014. The New Weight-Loss Drugs, Lorcaserin and Phentermine-Topiramate: Slim Pickings? *JAMA internal medicine*.

WOOLF, C. J. & MA, Q. 2007. Nociceptors—noxious stimulus detectors. *Neuron,* 55**,** 353-364.

WU, T., GAO, X., CHEN, M. & VAN DAM, R. M. 2009. Long-term effectiveness of diet-plus-exercise interventions vs. diet-only interventions for weight loss: a meta-analysis. *Obesity Reviews,* 10**,** 313-323.

YANG, H., YANG, S., CHAO, J. & CHEN, J. 2012. Beneficial effects of catechin-rich green tea and inulin on the body composition of overweight adults. *The British journal of nutrition,* 107**,** 749-54.

YANG, Z. & HALL, A. 2008. The financial burden of overweight and obesity among elderly Americans: the dynamics of weight, longevity, and health care cost. *Health services research,* 43**,** 849-868.

YANG, Z., WANG, X., WANG, H., HU, L., ZHENG, X. & LI, S. 2010. Capsaicin mediates cell death in bladder cancer T24 cells through reactive oxygen species production and mitochondrial depolarization. *Urology,* 75**,** 735-741.

YANNAKOULIA, M., MELISTAS, L., SOLOMOU, E. & YIANNAKOURIS, N. 2007. Association of eating frequency with body fatness in pre- and postmenopausal women. *Obesity,* 15**,** 100-6.

YANOVSKI, S. Z. & YANOVSKI, J. A. 2014. Long-term Drug Treatment for Obesity: A Systematic and Clinical Review. *JAMA : the journal of the American Medical Association,* 311**,** 74-86.

YATSUYA, H., FOLSOM, A. R., YAMAGISHI, K., NORTH, K. E., BRANCATI, F. L. & STEVENS, J. 2010. Race-and sex-specific associations of obesity measures with ischemic stroke incidence in the Atherosclerosis Risk in Communities (ARIC) study. *Stroke,* 41**,** 417-425.

YONESHIRO, T., AITA, S., KAWAI, Y., IWANAGA, T. & SAITO, M. 2012. Nonpungent capsaicin analogs (capsinoids) increase energy expenditure through the activation of brown adipose tissue in humans. *The American journal of clinical nutrition,* 95**,** 845-50.

YONESHIRO, T., AITA, S., MATSUSHITA, M., KAYAHARA, T., KAMEYA, T., KAWAI, Y., IWANAGA, T. & SAITO, M. 2013. Recruited brown adipose tissue as an antiobesity agent in humans. *Journal of Clinical Investigation,* 123**,** 3404-3408.

YONESHIRO, T., AITA, S., MATSUSHITA, M., OKAMATSU-OGURA, Y., KAMEYA, T., KAWAI, Y., MIYAGAWA, M., TSUJISAKI, M. & SAITO, M. 2011. Age‐Related Decrease in Cold‐Activated Brown Adipose Tissue and Accumulation of Body Fat in Healthy Humans. *Obesity,* 19**,** 1755-1760.

YOSHIOKA, M., IMANAGA, M., UEYAMA, H., YAMANE, M., KUBO, Y., BOIVIN, A., ST-AMAND, J., TANAKA, H. & KIYONAGA, A. 2004. Maximum tolerable dose of red pepper decreases fat intake independently of spicy sensation in the mouth. *The British journal of nutrition,* 91**,** 991-5.

YOSHIOKA, M., LIM, K., KIKUZATO, S., KIYONAGA, A., TANAKA, H., SHINDO, M. & SUZUKI, M. 1995. Effects of red-pepper diet on the energy metabolism in men. *Journal of nutritional science and vitaminology,* 41**,** 647-56.

YOSHIOKA, M., ST-PIERRE, S., DRAPEAU, V., DIONNE, I., DOUCET, E., SUZUKI, M. & TREMBLAY, A. 1999. Effects of red pepper on appetite and energy intake. *The British journal of nutrition,* 82**,** 115-23.

YOSHIOKA, M., ST-PIERRE, S., SUZUKI, M. & TREMBLAY, A. 1998. Effects of red pepper added to high-fat and high-carbohydrate meals on energy metabolism and substrate utilization in Japanese women. *The British journal of nutrition,* 80**,** 503-10.

YOUNG, J., SAVILLE, E., ROTHWELL, N., STOCK, M. & LANDSBERG, L. 1982. Effect of diet and cold exposure on norepinephrine turnover in brown adipose tissue of the rat. *Journal of Clinical Investigation,* 69**,** 1061.

YU, C. 2011. Study on HIF-1α gene translation in psoriatic epidermis with the topical treatment of capsaicin ointment. *ISRN pharmaceutics,* 2011.

YULIANA, N. D., KORTHOUT, H., WIJAYA, C. H., KIM, H. K. & VERPOORTE, R. 2014. Plant-derived food ingredients for stimulation of energy expenditure. *Critical reviews in food science and nutrition,* 54**,** 373-388.

ZEMEL, M. B., THOMPSON, W., MILSTEAD, A., MORRIS, K. & CAMPBELL, P. 2004. Calcium and dairy acceleration of weight and fat loss during energy restriction in obese adults. *Obesity Research,* 12**,** 582-590.

ZHANG, G., BAI, H., ZHANG, H., DEAN, C., WU, Q., LI, J., GUARIGLIA, S., MENG, Q. & CAI, D. 2011. Neuropeptide exocytosis involving synaptotagmin-4 and oxytocin in hypothalamic programming of body weight and energy balance. *Neuron,* 69**,** 523-535.

ZHANG, L., FANG, G., ZHENG, L., CHEN, Z. & LIU, X. 2013. Hypocholesterolemic Effect of Capsaicinoids in Rats Fed Diets with or without Cholesterol. *Journal of agricultural and food chemistry,* 61**,** 4287-4293.

ZHANG, L., LIU, D., MA, L., LUO, Z., CAO, T., ZHONG, J., YAN, Z., WANG, L., ZHAO, Z. & ZHU, S. 2007. Activation of transient receptor potential vanilloid type-1 channel prevents adipogenesis and obesity. *Circulation research,* 100**,** 1063-1070.

ZHOU, Z., PENG, J., WANG, C.-J., LI, D., LI, T.-T., HU, C.-P., CHEN, X.-P. & LI, Y.-J. 2010. Accelerated senescence of endothelial progenitor cells in hypertension is related to the reduction of calcitonin gene-related peptide. *Journal of hypertension,* 28**,** 931-939.

ZIMMET, P. & ALBERTI, K. 2012. Surgery or medical therapy for obese patients with type 2 diabetes. *N Engl J Med,* 366**,** 1635-1636.

Appendices

Appendix 1 Participant Information and forms for the intervention trial

Information Sheet

Study Title: The effects of Chilli Capsaicinoids on Weight Loss Markers in Females

Introduction

Research suggests capsaicinoids (found in chillies) may help people who are trying to lose weight and we wish to see the effects on people's body composition when they consume capsaicinoids over a period of time. You are being invited to take part in this study, but before you agree it's important you understand what it will involve for you.

What exactly will be happen if I take part in the study?

Pre-study screening questionnaire – After reading this form if you are interested in taking part you will ask you to complete a questionnaire. Participants for this study must be female, aged 18-45 years, without any medical conditions that can affect body weight, metabolism or digestion; pre-menopausal and not pregnant.

Consent – If you meet the inclusion criteria and would like to take part you must complete a consent form; however, you are free to withdraw at any time.

Which group? – To compare the results of our study some people will take a capsaicinoid supplement and some will take a placebo supplement (containing no active ingredients). To make sure the groups are the same you will be put into a group by chance (randomly). This will be a double blind trial so neither you nor the researchers will know which group you are in.

Measurements and blood samples – For this trial we will need to take a number of your measurements; this will include you're: Height, Weight, Waist and hip circumference, percentage body fat and a blood sample (done with a finger prick). These measurements and blood samples will be taken at the start, after 3 and 6 weeks of daily supplementation then one last time 3 weeks later.

Location - We would need you to come to Manchester Metropolitan University's physiology lab in the Hollings building (room C.G.29) on the All Saints campus on at the beginning of the study, after three weeks, six weeks and after eight weeks, so we could perform these measurements.

Supplements – You will be given six weeks' worth of supplements at the start of the trial; you will need to take two supplements a day.

Food and compliance diaries – You will be asked record what food and drinks you have consumed for three days of each week of the trial and complete a diary confirm you took the supplement each day.

What risks will I face in this study?

Capsaicinoids are a natural substance and in previous use of them in research the only reported side-effects was mild discomfort of the digestive system, although this only occurred at a higher dosage than the one being used in this trial.

Do I have to change my diet for the study?

We would like to maintain your normal diet and exercise habits throughout the study.

How can I benefit if I take part in this study? How could others benefit?

Benefits may include losing weight or body fat; however, we cannot promise that you will experience any benefits from participating in this study. We hope the information learned from this study will benefit others in the future.

If I wish to stop taking part in this study, what should I do?

You are free to leave the study at any time. If you are thinking of leaving the study, please contact Stephen Whiting (stephenjohnwhiting@gmail.com).

If I join this study, will it cost me anything?

You do not have to pay anything to be in this study. However, taking part in this study may lead to added costs for you such as travel expenses to the University.

How will my privacy be protected?

We take your confidentiality very seriously; your personal information will not be made public or shared with anyone and will be kept in accordance with the 1998 Data Protection Act. We are hoping to publish the results of the trial in a scientific journal, however all results will be published anonymously.

Who can I contact about this study?

If you have any questions or concerns before, during or after the study please contact Stephen Whiting at stephenjohnwhiting@gmail.com.

ID Nº:

Consent Form

	Please answer yes/no
Have you read the information sheet?	
Do you understand what the project is about?	
Have you asked all the questions you need too?	
Have all your questions been answered?	
Are you aware you will need to take a supplement every day?	
Are you aware that 4 blood samples will be taken and analysed?	
Are you aware a number of body measurements will be taken?	
Are you aware you will be asked to complete a weekly food diary?	
Are you aware you will be asked to complete a supplement diary?	
Can you confirm you are not pregnant?	
Do you understand that it is OK to stop taking part at any time?	

Are you willing to take part?

Name of participant	Date	Signature
Name of researcher	Date	Signature

ID Nº:

Screening Questionnaire

Part 1

Name	

Age	

Please state your ethnicity (please circle or delete as appropriate)	White Asian/Asian British British Arab/Middle Eastern	Mixed Race Black/Black Other

How would you describe your activity level at work? (please circle or delete)	Active active Moderately sedentary Sedentary	Moderately

How would you describe your activity level outside of work? (please circle or delete)	Active active Moderately sedentary Sedentary	Moderately

Please state your income level	£0 - £15,000

(please circle or delete as appropriate)	£15,000 - £25,000 £25,000 - £50,000 £50,000 - £100,000 £100,000+

Please state your occupation	

Please indicate your alcohol intake level. (please circle or delete) *1 pint of beer is 2 units* *1 large glass of wine is 3 units* *1 measure of spirits is 1 unit*	0-10 units per week 10-15 units per week 15-21 units per week 21-29 units per week 30+ units per week

How many of the following drinks do you drink a day? (please state a number)	Tea: Coffee: Caffeinated soft drinks (such as coke): Energy drinks (such as Red Bull):

Are you a smoker? (please circle or delete)	Yes No

Can you confirm you are not pregnant or planning to start a pregnancy during the course of the trial? (please circle or delete)	I not pregnant and have no plans to start a pregnancy I am pregnant or plan to start a pregnancy in the next 8 weeks

Can you confirm you have not yet started the menopause? (please circle or delete)	Not started	Have started

Do you follow a special diet or have any specific dietary requirements; such as vegetarian or vegan (if yes please state)	Yes	No

Have you lost of gained a large amount of weight in the last 6 months? (If yes, please give details)	Yes	No

Do you regularly take any supplements? (if yes please list)	Yes	No

Do you take any medication that may affect your weight such as Corticosteroids or Paxil? (if yes please list)	Yes	No

Do you suffer from any medical conditions that can affect your body weight, metabolism or digestion; such as Crohn's or coeliac disease?	Yes	No

Part 2

Please answer these questions by circling Y or N or delete as appropriate

I usually eat too much at social occasions, like parties and picnics	**Y**	**N**
Dieting is so hard for me because I just get too hungry	**Y**	**N**
I deliberately take small helpings as a means of controlling my weight	**Y**	**N**
Sometimes things just taste so good that I keep on eating even when I am no longer hungry	**Y**	**N**
When I feel anxious, I find myself eating	**Y**	**N**
Life is too short to worry about dieting	**Y**	**N**
I often feel so hungry that I just have to eat something	**Y**	**N**
It is not difficult for me to leave something on my plate	**Y**	**N**
When I am with someone who is overeating, I usually overeat too	**Y**	**N**
While on a diet, if I eat food that is not allowed, I consciously eat less for a period of time to make up for it	**Y**	**N**
When I feel unhappy, I often overeat	**Y**	**N**
I enjoy eating too much to spoil it by counting calories or watching my weight	**Y**	**N**
My weight has hardly changed at all in the last ten years	**Y**	**N**

I am always hungry so it is hard for me to stop eating before I finish the food on my plate	**Y**	**N**
When I feel lonely, I console myself by eating	**Y**	**N**
I eat anything I want, any time I want	**Y**	**N**
I count calories as a conscious means of controlling my weight	**Y**	**N**
I do not eat some foods because they make me fat	**Y**	**N**
I am always hungry enough to eat at any time	**Y**	**N**
While on a diet, if I eat a food that is not allowed, I often then splurge and - eat other high calorie foods	**Y**	**N**

Food Diary

Please record all the food and drinks you have consumed for 3 days (2 weekdays and one weekend day), in the space that follows. Please include as much detail as possible, including:

1. Approximate time of eating or drinking

2. Quantity or amount eaten e.g. 6 tablespoons, 1 cup, 1 thick slice, etc.

3. Type of food, e.g. orange juice, chocolate, chicken breast

4. Any brands names of products used, e.g. Philadelphia cream cheese, Kellogg's cornflakes cereal etc.

5. Addition of ingredients during preparation and cooking, e.g. sugar / oil

6. Any sub-recipes, e.g. for a sandwich may include 2 slices Warburton's medium sliced white bread (buttered), 50g of mature cheddar cheese, 2 slices wafer thin ham, 3 slices of tomato

7. Details of all drinks consumed

8. Please fill in the date at the top of each day you record.

9. We only need to know what you actually ate. You should not feel embarrassed about any food, as there are no "good" or "bad" foods. No one eats just the right foods all the time.

Over the page is an example day has been filled in for you to see.

Day 1 – Date: 06.03.2014 **ID N°: 024**

Time	Details of food and drink	Quantity eaten or description of size
8.30am	Toast – Hovis white bread Margarine Robinsons raspberry jam White coffee, 1 sugar	2 thick slices Thinly spread Thickly spread 1 mug
11am	Danish pastry – apple flavour Tea with milk, 1 sugar	1 medium
1.30pm	Ham and cheese sandwich: 30g cheddar cheese, 2 slices wafer thin ham, 2 slices Hovis white bread (margarine) White coffee, 1 sugar	1 sandwich 1 mug
4pm	Kit-kat Tea with milk 1 sugar	1 two finger 1 mug
7pm	Spaghetti Bolognaise, with onion, carrot and beef mince Orange squash	1 medium portion 1 medium glass
9.30pm	Tea with milk, 1 sugar	1 mug
10pm	Kelloggs crunchy nut cornflakes Semi-skimmed milk	1 large bowl
Any other notes *e.g. today was representative of a typical day, I was unwell today etc.*	This was a typical weekday	

ID Nº:

Day 1 – Date:

Time	Details of food and drink	Quantity eaten or description of size

Any other notes

e.g. today was representative of a typical day, I was unwell today etc.

Day 2 – Date:

Time	Details of food and drink	Quantity eaten or description of size

Any other notes
e.g. today was representative of a typical day, I was unwell today etc.

Day 3 – Date:

Time	Details of food and drink	Quantity eaten or description of size

Any other notes
e.g. today was representative of a typical day, I was unwell today etc.

Start Date:

ID Nº:

Pill Diary

1 **Supplement 1** ☐ **Supplement 2** ☐	**2** **Supplement 1** ☐ **Supplement 2** ☐	**3** **Supplement 1** ☐ **Supplement 2** ☐	**4** **Supplement 1** ☐ **Supplement 2** ☐	**5** **Supplement 1** ☐ **Supplement 2** ☐
6 **Supplement 1** ☐ **Supplement 2** ☐	**7** **Supplement 1** ☐ **Supplement 2** ☐	**8** **Supplement 1** ☐ **Supplement 2** ☐	**9** **Supplement 1** ☐ **Supplement 2** ☐	**10** **Supplement 1** ☐ **Supplement 2** ☐
11 **Supplement 1** ☐ **Supplement 2** ☐	**12** **Supplement 1** ☐ **Supplement 2** ☐	**13** **Supplement 1** ☐ **Supplement 2** ☐	**14** **Supplement 1** ☐ **Supplement 2** ☐	**15** **Supplement 1** ☐ **Supplement 2** ☐
16 **Supplement 1** ☐ **Supplement 2** ☐	**17** **Supplement 1** ☐ **Supplement 2** ☐	**18** **Supplement 1** ☐ **Supplement 2** ☐	**19** **Supplement 1** ☐ **Supplement 2** ☐	**20** **Supplement 1** ☐ **Supplement 2** ☐
21 **Supplement 1** ☐ **Supplement 2** ☐	**22** **Supplement 1** ☐ **Supplement 2** ☐	**23** **Supplement 1** ☐ **Supplement 2** ☐	**24** **Supplement 1** ☐ **Supplement 2** ☐	**25** **Supplement 1** ☐ **Supplement 2** ☐

26 **Supplement 1** ☐ **Supplement 2** ☐	**27** **Supplement 1** ☐ **Supplement 2** ☐	**28** **Supplement 1** ☐ **Supplement 2** ☐	**29** **Supplement 1** ☐ **Supplement 2** ☐	**30** **Supplement 1** ☐ **Supplement 2** ☐
31 **Supplement 1** ☐ **Supplement 2** ☐	**32** **Supplement 1** ☐ **Supplement 2** ☐	**33** **Supplement 1** ☐ **Supplement 2** ☐	**34** **Supplement 1** ☐ **Supplement 2** ☐	**35** **Supplement 1** ☐ **Supplement 2** ☐
36 **Supplement 1** ☐ **Supplement 2** ☐	**37** **Supplement 1** ☐ **Supplement 2** ☐	**38** **Supplement 1** ☐ **Supplement 2** ☐	**39** **Supplement 1** ☐ **Supplement 2** ☐	**40** **Supplement 1** ☐ **Supplement 2** ☐
41 **Supplement 1** ☐ **Supplement 2** ☐	**42** **Supplement 1** ☐ **Supplement 2** ☐			

Appendix 2 – Process of Ethical Approval

Application Number_____ Date Received_____

Application for Ethical Approval

Introduction

All university activity must be reviewed for ethical approval. In particular, all undergraduate, postgraduate and staff research work, projects and taught programmes must obtain approval from the Academic Ethics committee.

Application Procedure

The form should be completed legibly (preferably typed) and, so far as possible, in a way which would enable a layperson to understand the aims and methods of the research. Every relevant section should be completed. Applicants should also include a copy of any proposed advert, information sheet, consent form and, if relevant, any questionnaire being used. The Principal Investigator should sign the application form. Supporting documents, together with one copy of the full protocol should be sent to the Faculty/Campus Research Group Officer.

Your application will require external ethical approval by an NHS Research Ethics Committee if your research involves staff, patients or premises of the NHS (see guidance notes)

Work with children and vulnerable adults

You will be required to have an Enhanced CRB Disclosure, if your work involves children or vulnerable adults.

The Academic Ethics Committee will respond as soon as possible, and where appropriate, will operate a process of expedited review.

Applications that require approval by an NHS Research Ethics Committee or a Criminal Disclosure will take longer.

1. Details of Applicants

1.1. Name of applicant (Principal Investigator): Stephen Whiting

Telephone Number: 07834705020

Email address: 06437832@stu.mmu.ac.uk

Status: Postgraduate Researcher Postgraduate Student (Taught or Research)

Staff Department/School/Other Unit: Department of Food and Tourism Management

Programme of study (if applicable): n/a

Name of supervisor/Line manager: Kritika Mahadevan

1.2. Co-Workers and their role in the project: (e.g. students, external collaborators, etc.)

Name: Emma Derbyshire

2. Details of the Project

2.1. Title: Effects of chilli capsaicinoids on the body composition of overweight females.

2.2. Description of the Project: (please outline the background and the purpose of the research project, 250 words max): See end of application

2.3. Describe what type of study this is (e.g. qualitative or quantitative; also indicate how the data will be collected and analysed). See end of application

2.4. Are you going to use a questionnaire?

YES (Please attach a copy)

2.5. Start Date / Duration of project: February, lasting 9 weeks

2.6. Location of where the project and data collection will take place: MMU All Saints campus

2.7. Nature/Source of funding: MMU

2.8. Are there any regulatory requirements?

NO

3. Details of Participants

3.1. How many? 30 This sample size is based on a similar type of trial (Beneficial effects of catechin-rich green tea and inulin on the body composition of overweight adults; Yang et al Brit J Nutr (2012) 107, 749–754).

3.2. Age: 18 - 50

3.3. Sex: Female

3.4. How will they be recruited? Email, copy attached

3.5. Status of participants: (e.g. students, public, colleagues, children, hospital patients, prisoners, including young offenders, participants with mental illness or learning difficulties.) Students and university employees

3.6. Inclusion and exclusion from the project: see end of application

3.7. Payment to volunteers: (indicate any sums to be paid to volunteers). No payments

3.8. Study information: A copy of the information sheet is attached.

Have you provided a study information sheet for the participants?

YES (Please attach a copy)

3.9. Consent:

(A written consent form for the study participants MUST be provided in all cases, unless the research is a questionnaire.)

Have you produced a written consent form for the participants to sign for your records? YES (Please attach a copy)

4. Risks and Hazards

4.1. Are there any risks to the researcher and/or participants?

(Give details of the procedures and processes to be undertaken, e.g., if the researcher is a lone-worker.) See end of application

4.2. State precautions to minimise the risks and possible adverse events: See end of application

4.3. What discomfort (physical or psychological) danger or interference with normal activities might be suffered by the researcher and/or participant(s)? State precautions which will be taken to minimise them: n/a

5. Ethical Issues

5.1. Please describe any ethical issues raised and how you intend to address these: See end of document

6. Safeguards/Procedural Compliance

6.1. Confidentiality:

6.1.1. Indicate what steps will be taken to safeguard the confidentiality of participant records. If the data is to be computerised, it will be necessary to ensure compliance with the requirements of the Data Protection Act 1998.

6.1.2. If you are intending to make any kind of audio or visual recordings of the participants, please answer the following questions: n/a

6.1.2.1. How long will the recordings be retained and how will they be stored? n/a

6.1.2.2. How will they be destroyed at the end of the project? n/a

6.1.2.3. What further use, if any, do you intend to make of the recordings? n/a

6.2. The Human Tissue Act

The Human Tissue Act came into force in November 2004, and requires appropriate consent for, and regulates the removal, storage and use of all human tissue.

6.2.1. Does your project involve taking tissue samples, e.g., blood, urine, hair etc., from human subjects?

NO

6.2.2. Will this be discarded when the project is terminated?

N/A

6.3. Insurance

The University holds insurance policies in place to cover claims for negligence arising from the conduct of the University's normal business, which includes research carried out by staff and by undergraduate and postgraduate students as part of their course. This does not extend to clinical negligence.

In addition, the University has provision to award indemnity and/or compensation in the event of claims for non-negligent harm. This is on the condition that the project is accepted by the insurers prior to the commencement of the research project and approval has been granted for the project from a suitable ethics committee.

Research which is applicable to non-negligent harm cover involves humans and physical intervention which could give rise to a physical injury or illness which is outside the participants' day to day activities. This includes strenuous exercise, ingestion of substances, injection of substances, topical application of any substances, insertion of instruments, blood/tissue sampling of participants and scanning of participants.

The following types of research are not covered automatically for non-negligent harm if they are classed as the activities above and they involve:

Anything that assists with and /or alters the process of contraception, or investigating or participating in methods of contraception

Anything involving genetic engineering other than research in which the medical purpose is treating or diagnosing disease

Where the substance under investigation has been designed and /or manufactured by MMU

Pregnant women

Drug trials

Research involving children under sixteen years of age Professional sports persons and or elite athletes.

Overseas research

Will the proposed project result in you undertaking any research that includes any of the 8 points above or would not be considered as normal University business? If so, please detail below: No

6.4. Notification of Adverse Events (e.g., negative reaction, counsellor, etc.): (Indicate precautions taken to avoid adverse reactions.)

Please state the processes/procedures in place to respond to possible adverse reactions.

n/a

2.2 Description of the Project

Capsaicinoids (the ‘spicy’ chemicals found in chillies) have been proposed to have an anti-obesity effect (Whiting et al., 2012). Current rates of overweight and obesity are rising, in 2009, 1.5 billion adults (20 and older) were overweight; of these around 500 million were obese (World Health 2012). This has led to numerous health problems; obesity, especially visceral obesity carries a strong risk of metabolic diseases and related cardio-vascular disease.

Lifestyle modifications are important but reliance on diet and exercise interventions alone have proved relatively unsuccessful, particularly in the long term (Wu et al 2009). The use of bioactive ingredients in conjunction with weight loss programmes may help to further stimulate weight loss and lead to improvements in body composition profile (Belza et al 2007). Potentially helping people lose weight and maintain those gains.

While studies have looked at the anti-cancer (Yang et al 2010), anti-inflammatory (Choi et al 2011) and antioxidant (Henning et al 2011) effects of capsaicinoids, few have presented investigated potential weight loss benefits. The aim of this study will be to investigate whether capsaicinoid supplements affects body weight and fat mass in obese and overweight females.

2.3 Type of Study

The trail will be a quantitative study. A number of anthropometric measurements to be taken: height, body weight, waist circumference, hip circumference and body fat ratio (to be measured using bio-electrical impedance). The measurements will be recorded at baseline, week three, week six and week eight.

Blood samples (finger prick) will be collected at baseline, third, sixth and eighth week of the experiment; with blood glucose and blood lipid levels to be measured.

3.6 Inclusion Criteria

- Females
- Aged 20-50 years
- BMI ≥20kg/m2.

Exclusion criteria

- Use of medication and supplements
- Postmenopausal women
- Metabolic diseases
- Digestive diseases

- Pregnant females

4.1 Risks and Hazards

The intervention has been used in a number of trials before without and major side effects; the only reported problems have been some intestinal discomfort when a high dosage has been used.

The taking of blood.

4.2 Precautions

We plan to use a lower dosage so that this problem is minimised.

Blood will be taken via a finger prick by trained phlebotomist Mark Kelly

5.1 Ethical Issues

The trial will be conducted according to the guidelines laid down in the Declaration of Helsinki.

All procedures are to be approved by the MMU Ethics Committee.

Written consent will be obtained from all subjects, after they have read through the information sheet and had the opportunity to ask a researcher any questions they may have.

6.1.1 Confidentiality

All computerised records will comply with the requirements of the Data Protection Act 1998.

Study Protocol

Effects of chilli capsaicinoids on the body composition of overweight females.

Rationale

Capsaicinoids/capsinoids have been proposed to have an anti-obesity effect (Whiting *et al.,* 2012). Rates of overweight and obesity are rising, in 2009, 1.5 billion adults (20 and older) were overweight; of these around 500 million were obese (World Health 2012) (WHO, 2011c) (WHO, 2011b) (WHO, 2011b) (WHO, 2011b) (WHO, 2011c) (WHO, 2011c). This has led to numerous health problems; obesity, especially visceral obesity carries a strong risk of metabolic diseases and related CVD. Lifestyle modifications are important but reliance on diet and exercise interventions alone have proved relatively unsuccessful (Wu et al 2009). The use of bioactive ingredients in conjunction with weight loss programmes may help to further stimulate weight loss and lead to improvements in body composition profile (Belza et al 2007). While studies have looked at the anti-cancer (Yang et al 2010), anti-inflammatory (Choi et al 2011) and antioxidant (Henning et al 2011) effects of capsaicinoids, few have presented investigated potential weight loss benefits.

Aim: To investigate whether a capsaicinoid supplement affects body weight and fat mass in obese and overweight females.

Proposed methods

Subjects

- Aim for 60 females, aged 20-45 years
- BMI ≥20kg/m^2.
- Subjects to be recruited by announcement at MMU

Exclusion criteria:

- Use of medication and supplements
- Postmenopausal women
- Metabolic diseases
- Digestive diseases

Before the experiment is conducted, the content, purpose and possible study risks will be explained to all subjects and written, informed consent obtained.

Study design

- To be conducted according to the guidelines laid down in the Declaration of Helsinki.
- All procedures to be approved by the MMU Ethics Committee.
- Written consent to be obtained from all subjects.
- Subjects to be assigned to either a control (n=30) or experimental group (n=30)
- The intervention will be for 6-weeks, plus another assessment at week 8.
- Compliance of supplements to be monitored/logged with a pre-established protocol.
- The consumption of other food and beverages containing capsaicinoids/capsinoids to be prohibited.
- Subjects to be advised to maintain their diet and physical activity levels.
- Subjects will record their dietary intakes for 3 days at baseline, 6 weeks and 9 weeks.

Anthropometric measurements

Height, body weight, waist circumference and hip circumference are to be measured at baseline, third, sixth and eighth week.

Body fat ratio is to be measured using bioelectrical impedance analysis method (using 'BodyStat 4000').

Outcomes: Body weight (kg), BMI changes, changes in fat mass (kg), changes in body mass (kg), changes in waist circumference (cm), changes in hip circumference (cm).

Blood sampling and analysis

Blood samples (finger prick) are to be collected at baseline, sixth and eighth week of the trial. Blood glucose to be measured using 'Analox GM7 analyser' and blood lipid levels to be measured using 'CardioChek-PA' .

Safety and tolerability

Any symptoms reported?

Note: the intervention lasts for six weeks.

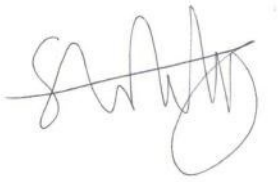
Participants to return week 9, to establish whether body weight/fat mass and biochemical parameters return back to normal (should increase back up in theory).

Confirmation of Ethical Amendments

Ethical approval was granted on 9th October 2013, since then the following amendments have been made to the trial:

- Placebo supplements to be filled by Stephen Whiting at MMU.
- Participants asked to make an extra visit to the university mid-way through the trial (to repeat the same measurements as other visits).
- Participants asked to complete a pill diary to confirm they have taken 2 supplements a day.
- Screening questionnaire created to assess eligibility for the trial and collect some basic participant data.

I can confirm I am supportive of these changes:



Stephen Whiting (Researcher)

Kritika Mahadevan (Supervisor)

Emma Derbyshire (Supervisor)

Appendix 3 – Publications

Systematic Review

Appetite 59 (2012) 341–348

Contents lists available at SciVerse ScienceDirect

Appetite

journal homepage: www.elsevier.com/locate/appet

Research review

Capsaicinoids and capsinoids. A potential role for weight management? A systematic review of the evidence

Stephen Whiting, Emma Derbyshire *, B.K. Tiwari

Manchester Food Research Centre, Manchester Metropolitan University, Hollings Faculty, Old Hall Lane, Manchester M14 6HR, UK

ARTICLE INFO

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Keywords:
Capsaicin
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Energy expenditure
Lipid oxidation

ABSTRACT

Capsaicinoids are a group of chemicals found in chilli peppers, with bioactive properties. The purpose of this study is to systematically review research investigating the potential benefits capsaicinoid compounds may have in relation to weight management. Medical databases were searched and 90 trials found, 20 of which were selected for inclusion, involving 563 participants. Three main areas of potential benefit for weight management were found: (1) increased energy expenditure; (2) increased lipid oxidation and (3) reduced appetite. Trial duration, dosage and sized varied, though trials were generally of high quality with a low risk of bias. It was observed that consumption of capsaicinoids increases energy expenditure by approximately 50 kcal/day, and that this would produce clinically significant levels of weight loss in 1–2 years. It was also observed that regular consumption significantly reduced abdominal adipose tissue levels and reduced appetite and energy intake. The mechanism of action is not presently fully understood, although it is well accepted much of the effects are caused by stimulation of the TRPV1 receptor. While capsaicinoids are not a magic bullet for weight loss, the evidence is that they could play a beneficial role, as part of a weight management program.

Contents

Introduction

Capsaicinoids are a group of molecules unique to fruits of plants from the genus *Capsicum* (chilli peppers). They are responsible for the fruit's pungent sensation and display potentially valuable pharmacological properties (Thiele, Mueller-Seitz, & Petz, 2008). This sensation occurs as capsaicin binds to the same group of nociceptors which also leads to the sensation of pain from heat and acid (Sanatombi & Sharma, 2008).

The basic chemical structure (Fig. 2) of the compounds is an acid amide of vanillylamine combined with a fatty acid (Aza-Gonzalez, Nunez-Palenius, & Ochoa-Alejo, 2011). Although more than 10 structures exist, the most prominent forms are

* Corresponding author.
E-mail address: e.derbyshire@mmu.ac.uk (E. Derbyshire).

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http://dx.doi.org/10.1016/j.appet.2012.05.015

Meta-Analysis Presentation (Nutrition Society Summer Conference)

Proceedings of the Nutrition Society (2012), **71 (OCE2)**, E169 doi:10.1017/S0029665112002261

Summer Meeting hosted by the Irish Section, 16–19 July 2012, Translational nutrition: integrating research, practice and policy

Is there Potential to use Bio-active Compounds (Capsaicinoids) as Innovative Weight Management Aids? A meta-analysis of evidence

S. Whiting, Dr E. J. Derbyshire and Dr. B. Tiwari

Food, Nutrition and Health Research Centre, Manchester Metropolitan University (MMU), Hollings Campus, Old Hall Lane, Manchester, M14 6HR, UK

We are in the midst of a global obesity crisis, which currently affects over 500 million adults in both industrialized and developing countries[1]. Although the health benefits of weight reduction are well-recognised, weight loss by diet and exercise fail in most patients[2]. Capsaicinoids are a group of natural chemicals found in chilli peppers that have bioactive properties which help to support weight management[3]. The aim of the present study was to conduct a meta-analysis studying the potential effects of capsaicinoids on appetite, which may, in turn, from the basis of a natural, safe weight loss aid.

Medical databases (Medline, Web of Knowledge and Scopus) were systematically searched for papers. Search terms were: 'capsaicin*' or chilli' and 'appetite'/'satiety'. Seven randomised control trials were found studying the effect on appetite, 4 of which provided results in format suitable to be combined in analysis. From the studies, 13 effect sizes were extracted and analysed.

Results showed that taking capsaicinoids prior to a meal reduced ad libitum EI by 393.95 KJ (94.09 Kcal) $p<0.001$ during the following meal. Although results should be viewed cautiously as heterogeneity was high ($I^2 = 80\%$).

Study findings suggest that regular consumption of capsaicinoids may contribute to weight management through reductions in EI. It has been shown that even small reductions in body weight (5%) can reduce obesity co-morbidities[4]. Larger and longer trials are now needed but there does appear to be some potential for capsaicinoids to be used as a natural weight-loss aid, particularly when used in conjunction with diet and exercise.

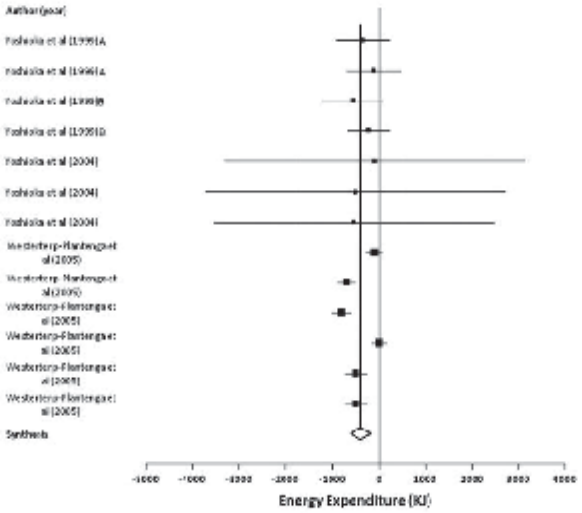

Fig. 1. Forest plot showing combined effect size.

1. Athenny CM (2004) *Obesity* **12**, 1191–1196.
2. World Health O (2011) *World health statistics* 2011.
3. Luo XJ, Peng J & Li YJ (2011) *Eur J Pharmacol* **650**, 1–7.
4. Seagle HM, Strain GW, Makris A *et al.* (2009) *J Am Diet Assoc* **109**, 330–346.

Meta-Analysis Paper

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Research review

Could capsaicinoids help to support weight management? A systematic review and meta-analysis of energy intake data ☆

S. Whiting [a], E.J. Derbyshire [a,*], B. Tiwari [b]

[a] School of Healthcare Science, John Dalton East Building, Oxford Road, Manchester M1 5GD, United Kingdom
[b] Teagasc Food Research Centre, Ashtown, Dublin 15, Ireland

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ABSTRACT

Objective: Capsaicinoids are a group of chemicals naturally occurring in chilli peppers with bioactive properties that may help to support weight management. The aim of the present study was to conduct a meta-analysis investigating the potential effects of capsaicinoids on energy intake, to clarify previous observations and form evidence-based conclusions about possible weight management roles. *Methods:* Medical databases (Medline, Web of Knowledge and Scopus) were systematically searched for papers. Search terms were: 'capsaicin*' or 'red pepper' or 'chilli*' or 'chili*' with 'satiety' or 'energy intake'. Of the seventy-four clinical trials identified, 10 were included, 8 of which provided results suitable to be combined in analysis (191 participants). From the studies, 19 effect sizes were extracted and analysed using MIX meta-analysis software. *Results:* Data analysis showed that capsaicinoid ingestion prior to a meal reduced *ad libitum* energy intake by 309.9 kJ (74.0 kcal) $p < 0.001$ during the meal. Results, however, should be viewed with some caution as heterogeneity was high ($I^2 = 75.7\%$). Study findings suggest a minimum dose of 2 mg of capsaicinoids is needed to contribute to reductions in *ad libitum* energy intake, which appears to be attributed to an altered preference for carbohydrate-rich foods over foods with a higher fat content. *Conclusions:* Meta-anlysis findings suggest that daily consumption of capsaicinoids may contribute to weight management through reductions in energy intake. Subsequently, there may be potential for capsaicinoids to be used as long-term, natural weight-loss aids. Further long-term randomised trials are now needed to investigate these effects.

Contents

Introduction

The plant of the genus capsicum produces a fruit (chilli pepper) with unique bioactive compounds (Kwon et al., 2011). The fruit contains a group of chemicals known as capsaicinoids, the most abundant and well known being capsaicin. Capsaicin, along with dihydrocapsaicin, makes up around 90% of capsaicinoids found in a typical chilli pepper (Meghvansi et al., 2010). These compounds are responsible for the fruit's 'pungent' flavour sensation that has

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* Corresponding author.
E-mail address: e.derbyshire@mmu.ac.uk (E.J. Derbyshire).

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