CAPSAICIN IN ATTENUATING METABOLIC SYNDROME

A dissertation submitted by

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Abstract

Capsaicin, the primary active constituent of chilli, has various recognised potential health benefits including analgesia but has also been investigated in studies focusing on obesity, hypertension, glucose impairment and altered blood lipid profiles. These physiological changes are the clustering of risk factors comprising the metabolic syndrome, which increases the risk of developing diabetes, non-alcoholic fatty liver disease and cardiovascular disease. Current clinical treatments demonstrate poor compliance and do not target the range of risk factors. Hence, the aim of this study is to determine whether low-dose dietary capsaicin attenuates the cardiovascular, liver and metabolic changes in a diet-induced rat model that mimics the human syndrome. 8-9 weeks old normotensive male Wistar rats were divided into 4 groups (n=8-12). Two groups were fed a cornstarch (C) diet and two were fed a high-carbohydrate, high-fat (H) diet, rich in saturated and *trans* fatty acids and simple sugars, thus mimicking the westernised-diet in humans. One group of each of the two diets was supplemented with dietary capsaicin (CC and HC; 13.3 and 7.3 mg/kg body weight/day) as a treatment for the final 8 weeks of the 16 week protocol. The H diet induced obesity, hypertension, dyslipidaemia, cardiovascular and liver damage and glucose intolerance by week 8 of the protocol. HC rats showed reduced abdominal circumference (24.3±0.5 cm to 19.0±0.2 cm), body weight gain (276±36 g to 186±9 g) and plasma concentrations of triglycerides (2.1±0.2 mmol/L to 1.4±0.2 mmol/L) compared to H rats. Capsaicin treatment reduced inflammation within the heart and liver, decreased hepatic fat vacuole enlargement and reduced cardiac collagen deposition. This decreased ventricular stiffness and further, reversed hepatic dysfunction by normalising liver damage in HC rats. Endothelial dysfunction and hypertension were normalised in HC rats, following a decrease in systolic blood pressure (153±2 mmHg to 132±2 mmHg). Finally, low-dose dietary capsaicin reversed glucose intolerance in H-fed rats. Hence, the present findings suggest that low-dose dietary capsaicin could be a potential therapeutic option for the reduction of obesity and metabolic syndrome in humans.

Key words – Capsaicin, transient receptor potential vanilloid 1(TRPV1), metabolic syndrome, cardiovascular, obesity, glucose, triglycerides, non-alcoholic fatty liver disease

Declaration

I hereby certify that the experimental work, results, analyses, discussion and conclusions reported in this dissertation are entirely my own effort, except where otherwise acknowledged. I also certify this work is original and has not been previously submitted.

Candidate: Edward Spencer Bliss (0050041459)

Date: 18th June 2017

Endorsement

Principal supervisor: Professor Lindsay Brown

Date: 18th June 2017

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List of abbreviations

TRP	Transient Receptor Potential
TRPV1	Transient Receptor Potential Vanilloid 1
CGRP	Calcitonin gene-related peptide
NKR	Neurokinin receptor
CALCRL	Calcitonin receptor-like receptor
ATP	Adenosine tri-phosphate
PKC	Protein kinase C
PKA	Protein kinase A
GPCR	G-protein coupled receptors
cAMP	Cyclic adenosine monophosphate
PLCβ	Phospholipase-Cβ
PIP_2	phosphatidylinositol-4,5-bisphosphate
CaMKII	Ca^{2+}/calmodulin dependent kinase II
PLCδ	Phospholipase-Cδ
RT-PCR	Reverse transcriptase polymerase chain reaction
eNOS	Endothelial nitric oxide synthase
HDL	High-density lipoprotein

LDL	Low-density lipoprotein
LDLR	Low-density lipoprotein receptor
LRP1	Low-density lipoprotein receptor related protein 1
ABCA1	Adenosine tri-phosphate-binding cassette transporter-A1
AMPK	Adenosine monophosphate activated protein kinase
IL6	Interleukin-6
TNFα	Tumour necrosis factor alpha
MCP1	Monocyte chemoattractant protein-1
MIP2	Macrophage inflammatory protein-2
COX2	Cyclooxygenase-2
PPARγ	Peroxisome proliferator-activated receptor gamma
GLP1	Glucagon-like peptide-1
BAT	Brown adipose tissue
RACGP	Royal Australian College of General Practitioners
NEFA	Non-esterified fatty acids
^{1}H-NMR	Proton nuclear magnetic resonance spectroscopy
PPM	Parts per million
H	High-carbohydrate, high-fat diet

C	Cornstarch deit
HC	high-carbohydrate, high-fat diet + capsaicin (5 mg/kg body weight/day)
CC	Cornstrach diet + capsaicin (5 mg/kg body weight/day)
BMI	Body mass index
ALT	alanine transaminase
AST	aspartate aminotransferase
SEM	Standard error of the mean
DXA	Dual-energy X-ray absorptiometer
OGTT	Oral glucose tolerance test
AUC	Area under the curve

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Chapter 1: Introduction and literature review

1.1 Metabolic syndrome and obesity

Metabolic syndrome is not described as a disease itself but rather as a constellation of symptoms that typically occur together (Harris 2013). There has been variation in both the definition and diagnosis of metabolic syndrome, but there is now international consensus that metabolic syndrome must constitute the presence of at least three of the following five conditions: central obesity, hypertension, decreased blood high density lipoproteins (HDL), elevated fasting glucose and elevated blood triglycerides concentrations (Harris 2013). These physiological changes enhance the risk of developing cardiovascular disease and type 2 diabetes and related complications, such as chronic renal disease (Harris 2013). Table 1 provides the criteria used by the Royal Australian College of General Practitioners (RACGP) in determining the diagnosis of metabolic syndrome in Australians.

Table 1: RACGP criteria for metabolic syndrome diagnosis.

Measure	Categorical Cut Point
Elevated waist circumference (measure of central obesity)	Population specific. However figure for Caucasian and Indigenous Australians: ≥102 cm in males and ≥88 cm in females
Elevated triglyceride levels	≥1.7 mmol/L
Reduced HDL	<1.0 mmol/L in men, <1.3 mmol/L in women
Elevated blood pressure	≥130 systolic or ≥85 diastolic
Elevated fasting glucose	>5.5 mmol/L

Reference: (Harris 2013) – modified.

The RACGP, using the criteria listed in Table 1, indicate that 20% of Australians have been diagnosed with metabolic syndrome since 2004 (Harris 2013). Furthermore, the RACGP identify obesity as the predominant factor leading to the metabolic syndrome diagnosis (Harris 2013). Obesity is one of the most rapidly-escalating epidemics faced by global public-health systems, in particular, those belonging to developed westernised societies, such as Australia. In the 1970s, overweight and obesity was uncommon with less than 15% of Australians being diagnosed in this

category (Hayes et al. 2017). By 1995, the rate of overweight and obesity had increased to approximately 20% and is expected to increase to 35% by 2025 (Tolhurst et al. 2016; Hayes et al. 2017). However, Australia currently possesses the highest incidence of overweight and obesity worldwide, affecting 63.4% of adults and 29.5% of people aged less than seventeen (Grima & Dixon 2013; Tolhurst et al. 2016). Additionally, 44.5% of adults and between 70.1-91.7% of people aged 17 or under do not meet the minimum daily physical activity requirements. More than 40% of the nation acquire their daily energy intake from 'junk' food, which is described as a westernised-diet high in both saturated fats and simple carbohydrates and, therefore, hyper-caloric (Tolhurst et al. 2016). Obesity occurs when there is increased fat deposition following an imbalance between energy consumption and expenditure, where consumption exceeds expenditure. However, extending from this simplistic definition, obesity is a consequence of multifaceted interactions among genetic, environmental, socio-economic, psychological and dietary factors, thus making obesity a complex disease to understand and combat (Moran & Shanahan 2014; Bauer et al. 2016).

Obesity is characterised by the presence of parameters indicating increased adiposity, low-grade inflammation, dysbiosis, increased neurogenic tone and hormonal imbalances (Buhmann et al. 2014; Moran & Shanahan 2014; Bauer et al. 2016). These obesogenic factors give rise to comorbidities (Table 2), which in turn increase morbidity and mortality. Therefore, obesity determinants, as well as the associated costs, which are in excess of $8 billion per year in Australia alone, and unsuccessful non-invasive treatment interventions have resulted in an increase in research aimed at decreasing weight-loss (Grima & Dixon 2013; Buhmann et al. 2014). Moreover, there is poor compliance with clinical treatments such as very low energy diets and pharmacotherapy, thus leaving bariatric surgery as the most effective option (Grima & Dixon 2013). Therefore, there is a need to find and evaluate effective and inexpensive treatments with enhanced compliance rates to decrease the momentum of this escalating problem. Since an increased energy intake provided by hypercaloric foods is a chief contributing factor to this epidemic, it seems counter-intuitive that foods may assist in combating the obesity issue. However,

many new drugs and pharmacological treatments are based on naturally-occurring molecules, for example, pseudoephedrine is a diastereomer of adrenaline while acetylsalicylic acid, or aspirin, is derived from the phytochemical salicylic acid extracted from the willow tree (Takenaka 2001; Desborough & Keeling 2017). Hence, searching for naturally-occurring bio-active compounds, especially within foodstuffs, is a logical step in finding effective treatments that may combat a range of pathological conditions including obesity.

Table 2: The health risks associated with overweight and obesity.

Body System	Health Risk
Cardiovascular	Stroke
	Coronary heart disease
	Cardiac failure
	Hypertension
Gastrointestinal	Non-alcoholic fatty liver disease
	Gallbladder disease
	Pancreatic disease
	Gastro-oesophageal reflux disease
	Cancers of the bowel, oesophagus, gall bladder and pancreas
Endocrine	Non-insulin dependent diabetes mellitus
	Polycystic ovary syndrome
Genitourinary	Chronic kidney disease, which can lead to end-stage renal disease
	Cancers of the kidney and prostate
	Kidney stones
	Stress urinary incontinence (women)
	Sexual dysfunction (men)
Pulmonary	Obstructive sleep apnoea
	Obesity hypoventilation syndrome
	Asthma
Musculoskeletal	Osteoarthritis – especially the patella
	Spinal disc disorders and lower back pain
	Disorders of soft tissue structures such as tendons, fascia and cartilage
	Foot pain
	Mobility disability
Reproductive	Menstrual disorders
	Miscarriage and poor pregnancy outcome
	Infertility/sub-fertility
	Cancers of the breast (postmenopausal women), endometrium and ovaries
Mental/Psychological	Depression
	Eating disorders
	Reduced health-related quality of life

Reference: (Grima & Dixon 2013) – modified

1.2 Nutraceuticals and spices

A nutraceutical is defined as any substance that is a food or part of a food and provides medical or health benefits, including the prevention and treatment of disease (DeFelice 1995). Traditional medicines, such as Chinese medicine, folklore remedies and Indian Ayurveda, which all incorporate the use of natural products, have been practised for millennia (WHO 2000). The use of these products in traditional medicines incorporates a broad range of nutraceuticals, all with different efficacy and varying adverse effects (WHO 2000). Historically, these medicines were often mixed together to form a concoction and the recipes passed on from generation to generation, with various doses that were deemed effective and safe in treating an ailment (WHO 2000). Currently, nutraceuticals are typically extracts or parts of a food that are supplemented into the diet. Dietary supplementation allows a concentrated quantity or dose of an extract to be consumed readily by a consumer (DeFelice 1995). Whilst traditional medicines may provide insight into what products may have been effective and at what dose, current studies aim at determining the efficacy and therapeutic benefits, in addition to the potential adverse effects, that a nutraceutical may deliver.

Spices such as turmeric containing curcumin, black pepper containing piperine, garlic containing allicin and ginger containing zingerone and gingerol have been investigated in human and animal studies and acknowledged as potential nutraceuticals in the treatment of obesity and other symptoms of metabolic syndrome (Diwan et al. 2013; Saravanan et al. 2014; Matsumoto et al. 2016; Panahi et al. 2016). These studies indicate that spices reduce the chronic systemic low-grade inflammation evident in diet-induced obese patients, which, in turn, alleviates glucose impairment, dyslipidaemia, hypertension, non-alcoholic liver disease and other cardiovascular disease. However, these studies do not investigate the long-term effects and adverse effects associated with their usage. Hence, further studies are needed to determine the efficacy and possible adverse effects of chronic treatment. Hence, this study will evaluate the primary pungent phytochemical in chilli, capsaicin, as a treatment for metabolic syndrome in a diet-induced obese rat model.

1.2 Chilli – A Brief History

Chilli is a popular fruit commonly used to increase the flavour and piquancy of food. Evidence suggests chillies have been cultivated for domestic use in Mexico and Bolivia for 7000 years (Mortensen & Mortensen 2009; Kraft et al. 2014). In 1492, Columbus noted that the South Americans added chilli to food, whilst his physician noted the medicinal effects of chilli including folklore remedies to clean wounds, assist in bowel movement and 'purify' living quarters (Mózsik et al. 2009b; Guido et al. 2015).

Thresh (1876) isolated capsaicin, the primary active constituent of chilli, noting that it was a white crystalline, odourless and volatile compound. Nelson (1919) first determined the molecular structure of capsaicin (Figure 1). Capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homocapsaicin and homodihydrocapsaicin constitute the capsaicinoids (Figure 1) (Rollyson et al. 2014). Capsaicinoids are exclusively produced by the *Capsicum* genus and are found in abundance in the epidermal cells of the placenta in the pod of the fruit (Zahra et al. 2016). Capsaicin constitutes approximately 70-80% of the capsaicinoids found in any fruit belonging to the *Capsicum* genus, thus making it the primary and most abundantly occurring capsaicinoid (Zahra et al. 2016).

It was not until the 18th and 19th centuries that the chilli gained popularity in the Western world for its uses in folklore medicine. During this period, chilli was used predominantly as a rubefacient to induce analgesia and to assist in heat regulation in tropical regions (Abdel-Salam 2014; Fattori et al. 2016). Late 19th century studies with capsaicin as a pharmacological agent suggested that capsaicin acted on sensory nerves because capsaicin did not induce dermal vesiculation, enhanced motility of the gastrointestinal tract without gustatory effect and induced a fall in body temperature (Lee 1954; Abdel-Salam 2014).

Ninety years later, Szolcsányi (1977) demonstrated by measuring the response of sensory fibres from the cat saphenous nerve that capsaicin acts as an afferent blocking agent. Capsaicin induces stimulation of sensory fibres initially and later causes signal blockage of the fibres as well as the

warmth receptors (Abdel-Salam 2014; Fattori et al. 2016). Szolcsányi (1977) also measured the reduction in the thermal threshold of warmth receptors when capsaicin was applied to the epidermis of a rat or the human tongue. Szolcsányi (1977) confirmed the findings of late 19th century studies and also demonstrated that the response induced by capsaicin on sensory receptors was polymodal. This means that these sensory receptors (nociceptors) are responsive to mechanical, chemical and thermal stimuli (Abdel-Salam 2014). Furthermore, this started the study of capsaicin for pain relief due to the desensitising effect of capsaicin on mammalian polymodal nociceptors (Abdel-Salam 2014).

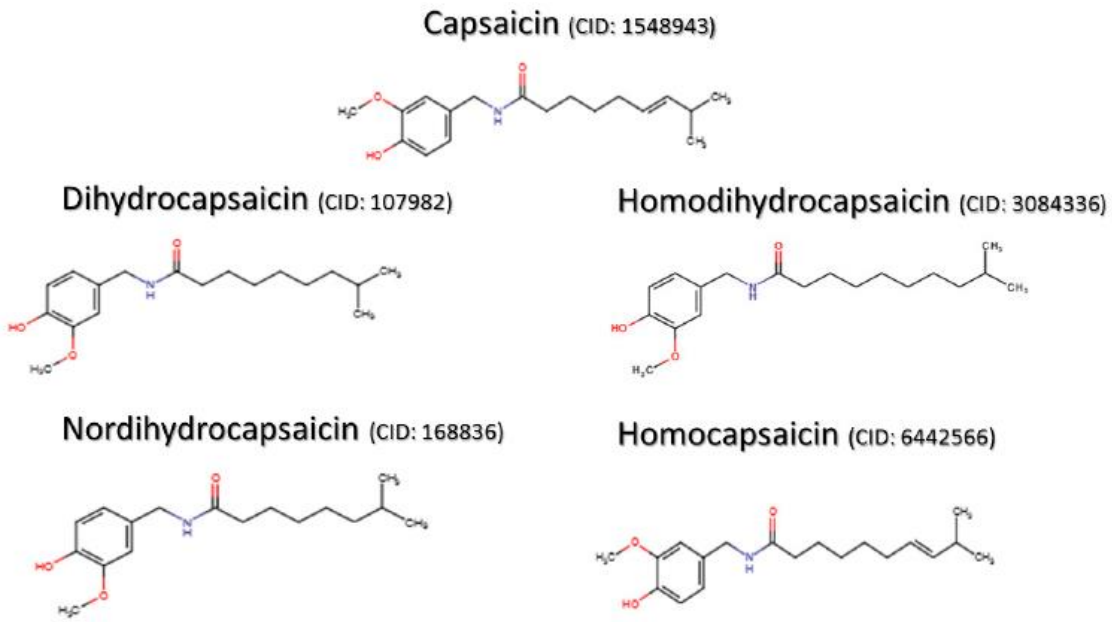


Figure 1: The chemical structure of capsaicin and the capsaicinoids. Note the common vanillyl ring present in each capsaicinoid (Fattori et al. 2016).

1.3 Nociception: foundations into determining the actions of capsaicin

Nociceptors are sensory neurons that possess the ability to detect noxious and potentially noxious stimuli from both internal and external stimuli (Yang et al. 2016). They process this pain-related information into electrical signals (transduction) which are subsequently conducted to the brain through different signalling pathways (Handwerker 2008; Reddi et al. 2013). These neurons are able to detect chemical, thermal and mechanical stimuli and are, therefore, termed polymodal (Szolcsányi 1977; Szolcsányi 2015; Yang et al. 2016).

Nociceptors can alter their neurophysiological properties when they are exposed to repetitive or continuous noxious stimuli (Abdel-Salam 2014). Yang et al. (2016) support the landmark study of Szolcsányi (1977) that demonstrated the ability of nociceptors to change their sensitivity to stimuli such as high-dose capsaicin. The findings of this study were that repetitive applications of topical capsaicin to a given site could cause a decrease in neuroplasticity due to afferent nerve withdrawal or death, sensitisation, and/or desensitisation. Hence, since this seminal study, topical capsaicin applications have been used extensively to understand how this pain-related information is transduced from the periphery to the central nervous system.

When capsaicin is applied topically to a given site it causes an influx of cations, such as calcium (Ca^{2+}), through plasmalemmal channels of nociceptors (Woolf 2011). This subsequently induces depolarisation and the initiation of an action potential, thus stimulating an afferent message of perceived pain via different pathways to the brain (Iannetti et al. 2013; Dezhdar et al. 2015). These pathways are detailed in Figures 2 and 3. Additionally, this was the premise used for isolating and identifying the molecular receptor that capsaicin binds to within nociceptors (Caterina et al. 1997). Caterina et al. (1997) were able to see an increased expression of a functional protein and consequently utilised a cDNA based strategy to clone the receptor. This was subsequently validated by demonstrating that the receptor, Transient Receptor Potential Vanilloid 1 (TRPV1), functioned *in vivo* when a nociceptor was stimulated with capsaicin (Caterina et al. 1997). This provided a mechanism for how capsaicin exerted its effects on the cellular level.

Whilst this explanation into nociception is simplistic, it can be appreciated that the central nervous system involvement in processing and discriminating between the types of stimuli is highly organised, developed and complexed and yet to be fully elucidated. Furthermore, an understanding of how these pathways function and how topical application of capsaicin elicits a response in the brain is important, as it provides the foundations of how capsaicin may link other peripheral sensory fibres expressing TRPV1, such as vagal afferent fibres in the gut, to the brain.

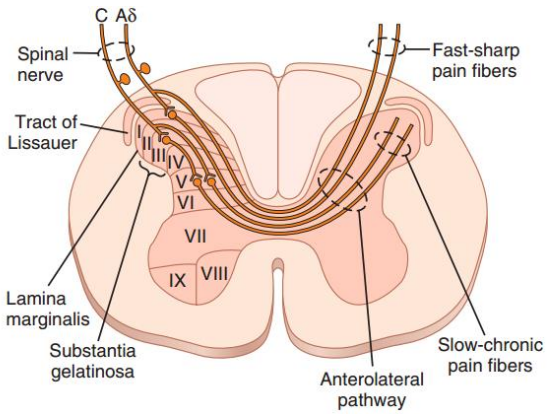


Figure 2: The dorsal horn and the spinal laminae. The interactions that occur within the laminae of the dorsal horn are intricate and involve the primary afferent signals, interneurons and the descending modulatory pathway. First order neurons that terminate in the horn include the myelinated Aδ fibres and unmyelinated C-fibres. The C-fibres predominantly terminate in the second and third laminae and are responsible for transmitting the slow, diffuse, burning pain associated with topical capsaicin application (Hall 2015).

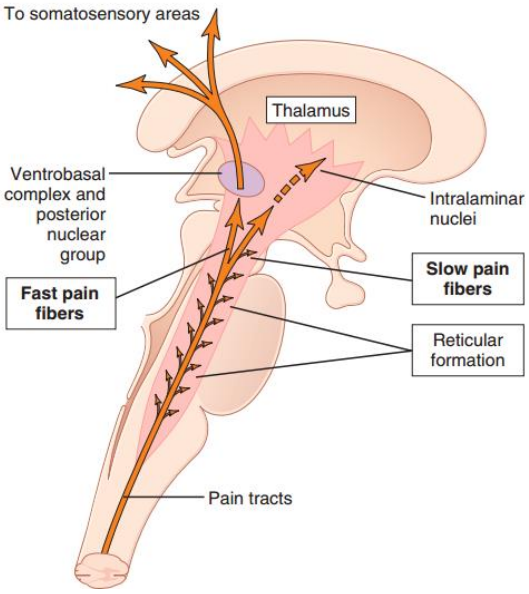


Figure 3: Pain transmission to the brain. Both the neospinothalamic and paleospinothalamic tracts are involved in the polymodal detection and processing of peripheral stimuli to the brain. The neospinothalamic tract is predominantly used for information being relayed by the Aδ fibres. The axons of these second order neurons decussate at the spinal midline and ascend to the thalamus as the lateral spinothalamic tract. From these thalamic areas, the signals are conducted to other basal areas of the brain as well as to the somatosensory cortex via third order neurons. The paleospinothalamic tract predominantly involves C-fibres. C-fibres synapse with dorsal horn interneurons, which subsequently synapse with second order neurons. The axons of the second order neurons mostly join the fibres from the neospinothalamic pathway. However these axons first pass through the anterior commissure to the opposite side of the spinal cord, proceeding upward to the anterolateral pathway. Majority of these neurons terminate in the reticular formation of the brain stem, whilst the minority of the fibres project to the thalamus (Hall 2015).

1.4 Transient Receptor Potential Vanilloid 1 – the capsaicin receptor

1.4.1 Transient Receptor Potential Superfamily

Transient Receptor Potential (TRPs) channels are among the most broad and diverse of the mammalian regulatory channels (O'Neill et al. 2012). Currently, there are 28 variants in the TRP family and these can be further divided into a variety of sub-groups depending on their function (Wu et al. 2010). They are non-selective cation channels located predominantly in the plasmalemma (Wu et al. 2010; Liao et al. 2013; Hanson et al. 2015). TRPs predominantly function as transducers in response to chemical and physical stimuli such as temperature, low pH, osmotic sensing, taste, pressure, stretch, vibration and endogenous and exogenous molecules (Wu et al. 2010; O'Neill et al. 2012; Liao et al. 2013; Hanson et al. 2015).

1.4.2 The Structure of TRPV1 and its agonists

TRPV1 is one of six membrane proteins that belong to the vanilloid subgroup of the TRP superfamily (Hudson et al. 2016). It is a non-selective cation channel conveying polymodal activation by an array of mechanisms, such as mechanical stimulation, pH, endogenous and exogenous cannabinoids, noxious peptide toxins, heat greater than 43˚C and, most importantly, by vanilloids such as capsaicin (Movahed et al. 2005; Dhaka et al. 2009; Bang et al. 2010; Hakim et al. 2015; Laing & Dhaka 2015; Zhang et al. 2015; Bae et al. 2016). Caterina et al. (1997) categorised TRPV1 as the capsaicin receptor, due to its activation by capsaicin and suggested that it is predominantly expressed in nociceptors. However, since this seminal study, it has been established that the TRPV1 is expressed abundantly throughout the body (Tominaga et al. 1998). Caterina et al. (1997) first isolated and cloned the 95 kilo Dalton protein and determined the TRPV1 gene to consist of 2514 nucleotides, which are subsequently transcribed and translated into a protein of 838 amino acids. The rat and human TRPV1 receptors only differ by one amino acid and are, therefore, considered structurally and functionally orthologous (Hayes et al. 2000). Liao et al. (2013) used electron cryo-microscopy to determine the structure of the TRPV1 channel as an integral membrane

protein and a homo-tetramer comprised of six transmembrane α-helices (S1-S6), two cytoplasmic components (N-terminus and C-terminus) and a central ion pathway. Finally Liao et al. (2013) observed that the TRPV1 channels contained the conserved TRPV cytoplasmic ankyrin repeat domain, which is postulated to facilitate TRPV1 subunit association (Cao et al. 2013a) (Figure 4). While determining how TRPV1 subunits function upon activation has proven difficult, further studies are needed to elucidate its polymodal functioning, as this may provide site-specific targets for the treatment of many pathophysiological conditions. Additionally, solving the allosteric properties of TRPV1 may assist in determining how capsaicin may sensitise and desensitise a cell following short- and long-term low-dose capsaicin administration.

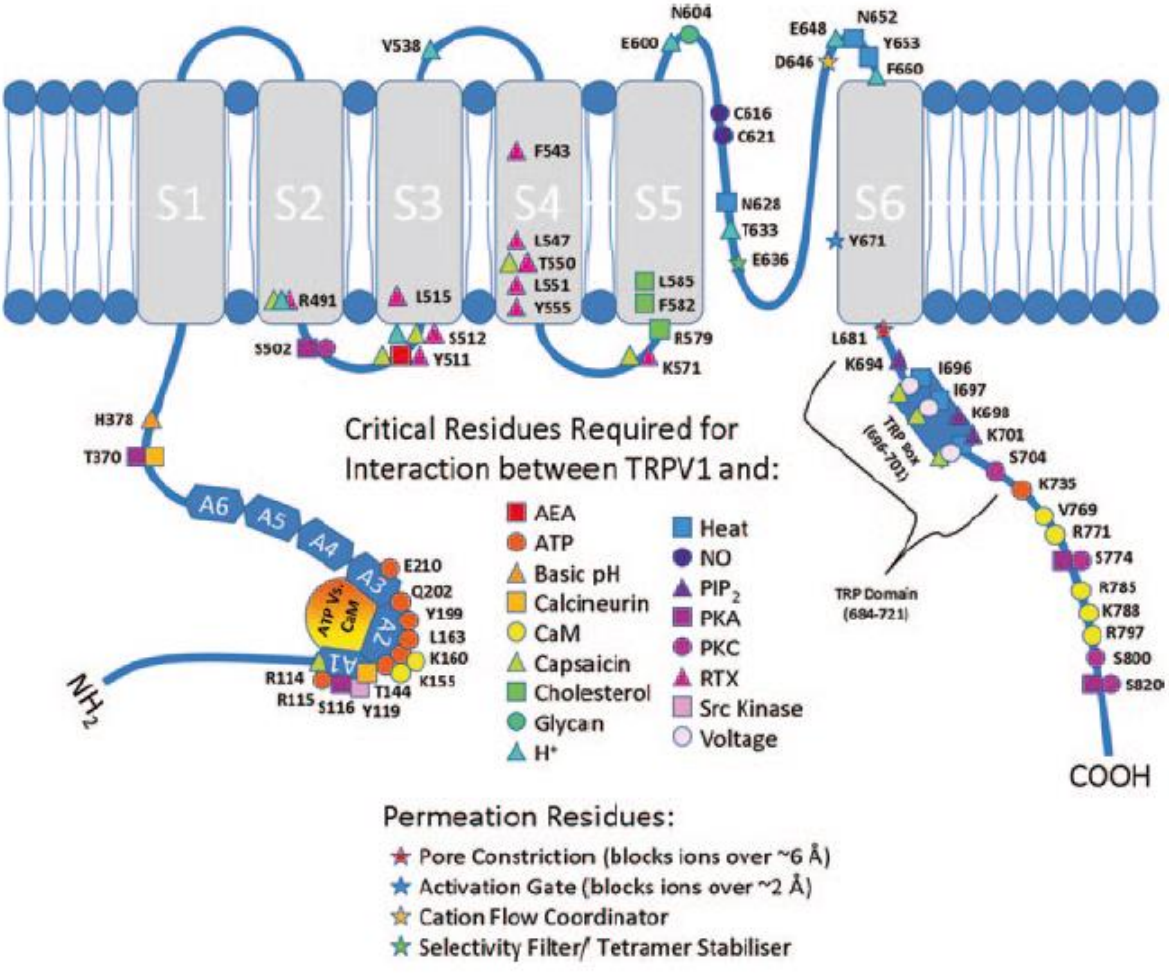


Figure 4: TRPV1 topology and the critical residues involved in TRPV1 function. The TRPV1 pore possesses a conical shape where it is wider extracellularly compared to its intracellular passage opening (Abdel-Salam 2014)

1.4.3 Capsaicin activation of TRPV1

When capsaicin binds to TRPV1, the channel undergoes conformational change and becomes permeable to cations such as Ca^{2+} (Abdel-Salam 2014). Capsaicin binds to the vanilloid-binding pocket, which is located on the intracellular side of the plasmalemma (Abdel-Salam 2014; Fattori et al. 2016; Hudson et al. 2016). Kumar et al. (2016) have postulated that the tyrosine residue Y511 is responsible for anchoring capsaicin to the vanilloid-binding pocket (see figure 4) thus augmenting the time that capsaicin complexes with TRPV1. This notion supports previous studies that suggest this may occur in both humans and rats as the receptors are orthologous (Cao et al. 2013a; Liao et al. 2013; Bae et al. 2016). However, given the other free amino acid residues, this may not be the only residue implicated. In any case, capsaicin allosterically alters TRPV1 properties, thus causing an opening of the pore and permitting cation influx, namely Ca^{2+} (Kumar et al. 2016). This influx causes a change in electrical properties of the cell, and induces depolarisation and increases the likelihood of an action potential occurring, releasing neurotransmitters such as substance P and calcitonin gene-related peptide (CGRP) by exocytosis (Devesa et al. 2014; Smutzer & Devassy 2016). Substance P facilitates nociception and induces vasodilatation via its interaction with the neurokinin receptor (NKR) in the endothelium of blood vessels (Devesa et al. 2014; Steinhoff et al. 2014). This also holds true for CGRP release, with the exception that it binds to calcitonin receptor-like receptor (CALCRL) in lieu of NKR (Russell et al. 2014; Steinhoff et al. 2014). This interaction subsequently increases vasculature permeability and permits localised granulocyte infiltration (Russell et al. 2014; Steinhoff et al. 2014). Furthermore, substance P binds to NKR and CGRP binds to CALCRL on mast cells, and these cells in conjunction with granulocytes and surrounding tissue induce the release of proinflammatory mediators such as histamine, nerve growth factor, bradykinin, prostaglandins and adenosine tri-phosphate (ATP) (Russell et al. 2014; Steinhoff et al. 2014; Smutzer & Devassy 2016). However, the release of CGRP and substance P decrease can also reduce inflammation, depending on the situation (Russell et al. 2014; Steinhoff et al. 2014). For example, both the sustained release and systemic administration of CGRP have been demonstrated

to inhibit monocyte chemoattractant protein-1 (MCP1) expression, a primary proinflammatory chemokine raised in various physiological conditions, such as obesity and non-alcoholic liver disease and inflammatory cell migration into regions such as the vasculature (Zhang et al. 2009; Zhang et al. 2010). Hence, further studies are needed to determine the factors that elicit both pro- and anti-inflammatory actions conveyed by substance P and, in particular, CGRP, as this may be the key in determining the anti-inflammatory properties of capsaicin upon activating TRPV1.

1.4.4 Capsaicin-induced TRPV1 Sensitisation

TRPV1 contains several phosphorylation sites/residues (Figure 4) (Abdel-Salam 2014). TRPV1 phosphorylation is typically associated with sensitisation, while TRPV1 dephosphorylation is typically associated with desensitisation (Vriens et al. 2009; Ho et al. 2012). TRPV1 becomes sensitised via phosphorylation by protein kinase C (PKC) or protein kinase A (PKA), with both being activated by G-protein coupled receptors (GPCR) (Vriens et al. 2009; Ho et al. 2012; Smutzer & Devassy 2016). When capsaicin activates TRPV1 as outlined above, prostaglandin E_2 and serotonin bind to their respective GPCR, which stimulates dissociation of the stimulatory G-protein subunit (G_s) from the GPCR (Vriens et al. 2009; Ho et al. 2012; Smutzer & Devassy 2016). When this occurs, adenylate cyclase is activated by the G_s to synthesise cyclic adenosine monophosphate (cAMP) (Vriens et al. 2009; Smutzer & Devassy 2016). Increased cytosolic cAMP concentrations signal PKA to phosphorylate TRPV1, which subsequently impedes dephosphorylation and prevents desensitisation, thus promoting an increased sensitivity to endogenous endovanilloids (Vriens et al. 2009; Cao et al. 2013a; Smutzer & Devassy 2016). However, further studies, in particular *in vitro* studies, are needed to determine this mechanism. Additionally, if serotonin release is stimulated by capsaicin-induced TRPV1 activation, it may act to assist in appetite regulation, as increased systemic concentrations of serotonin have been linked to increased satiety and weight loss (Savastano et al. 2007; Lam et al. 2008; Halford & Harrold 2012). Hence, further studies are needed to determine the link between capsaicin, increased serotonin release, satiety and, therefore, energy intake and weight loss.

When bradykinin and other cytokines are released, they bind to specific GPCRs and stimulate the dissociation of the G-protein subunit (G_q) from the receptor (Ho et al. 2012; Smutzer & Devassy 2016). G_q initiates the second messenger system by activating phospholipase-Cβ (PLCβ) to hydrolyse the phospholipid, phosphatidylinositol-4,5-bisphosphate, known as PIP_2 (Morales-Lázaro et al. 2013; Smutzer & Devassy 2016). PIP_2 is involved in cellular protein regulation, the anchoring of proteins to the plasmalemma and activating intracellular second messenger pathways (Morales-Lázaro et al. 2013). PIP_2 is cleaved into inositol triphosphate and diacylglycerol by PLCβ (Morales-Lázaro et al. 2013). Free diacylglycerol has a high affinity for PKC and consequently binds to PKC, thus stimulating its activation (Morenilla-Palao et al. 2004; Vriens et al. 2009; Morales-Lázaro et al. 2013). Both Vriens et al. (2009) and Por et al. (2013) suggest that TRPV1 phosphorylation by PKC sensitises it to chemical stimuli, thus lowering its activation threshold. This means that TRPV1 activation by capsaicin is more likely to result in a cellular response and, therefore, the release of various cytokines depending on the cell type in which it is expressed. However further studies are needed for this hypothesis to be validated and the exact mechanism to be elucidated. These sequences of events may provide insight in to how low-dose capsaicin elicits different responses throughout the body in various pathophysiological conditions, such as obesity. For example, it could by hypothesised that low-dose capsaicin, which binds to TRPV1-expressing vagal afferents in the gut, may result in increased sensitivity of these fibres to repeated doses of capsaicin, thus increasing the likelihood of vagal firing. Given that vagal fibres link the gut to the brain and their activation is linked to increased synthesis and release of gut-peptides, such as GLP1, which improves glucose utilisation and increases satiety resulting in a decreased energy intake, this may provide a mechanism as to how capsaicin exerts its anti-obesogenic and anti-hyperglycaemic effects (Buhmann et al. 2014). However, further studies linking the molecular and systemic physiology are needed to confirm if these theories are plausible.

1.4.5 TRPV1 desensitisation and modulation in response to capsaicin

Capsaicin's use as an analgesic appears contradictory, as repeated or extended stimulation of a nociceptor, or more specifically, TRPV1, leads to a decrease in the pain-evoking responses and TRPV1 activity (Abdel-Salam 2014). This is referred to as desensitisation and provides the foundations of capsaicin-induced analgesia (Abdel-Salam 2014). Figure 5 demonstrates an array of pathophysiological conditions associated with central sensitisation. Therefore, an understanding of sensitising and desensitising of the TRPV1 receptors is necessary to understand possible long-term changes to the responses to capsaicin in metabolic syndrome.

TRPV1 desensitisation is mediated by the influx of cytosolic Ca^{2+} from TRPV1 activation (Rohacs 2015). Mohapatra and Nau (2005) have demonstrated that desensitisation does not occur in the absence of Ca^{2+}. Furthermore, the homeostasis of Ca^{2+} is vital in cell survival, as increased intracellular Ca^{2+} concentrations are cytotoxic (Orrenius et al. 2015). Hence, TRPV1 activity must be regulated in order to relieve apoptosis (Orrenius et al. 2015).

1.4.6 Dephosphorylation of TRPV1

TRPV1 is modulated through changes in calmodulin activity (Smutzer & Devassy 2016). Calmodulin has a high affinity for Ca^{2+} and binds Ca^{2+} when available in excess (Rohacs 2015; Smutzer & Devassy 2016). Once calmodulin is activated, it stimulates the activity of calcineurin, which functions as a phosphatase and potentiates TRPV1 desensitisation (Rohacs 2015; Smutzer & Devassy 2016). While PKA phosphorylation acts to enhance TRPV1 sensitivity (Por et al. 2013), Mohapatra and Nau (2005) demonstrated that inhibition of calcineurin increased TRPV1 sensitivity to capsaicin. Calmodulin also activated Ca^{2+}/calmodulin dependent kinase II (CaMKII), which has been postulated by Jung et al. (2004) to phosphorylate TRPV1 at PKA and PKC residues and potentiate TRPV1 sensitisation. However, Por et al. (2013) have demonstrated that inhibition of this complex does not potentiate TRPV1 desensitisation. Therefore, more studies are needed to consider the role of CaMKII in TRPV1 physiology.

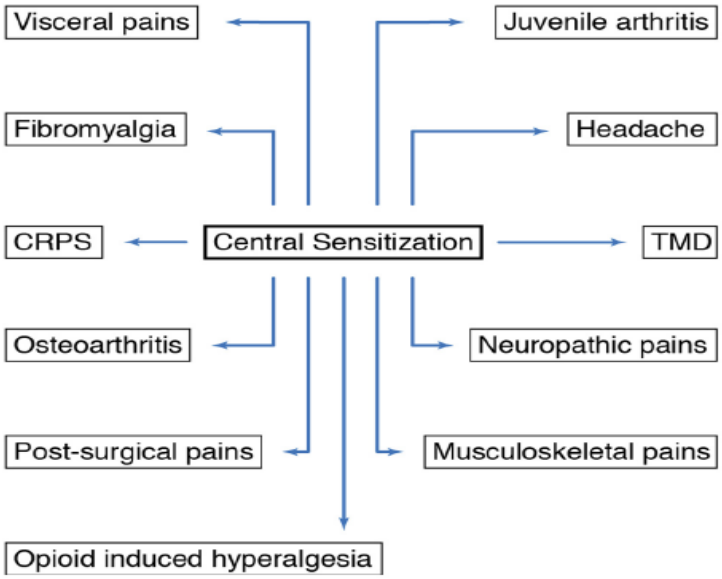


Figure 5: Pathophysiological pain conditions associated with central sensitisation that are relieved by capsaicin. Central sensitisation is thought to be a key phenomenon contributing to the development of these chronic pain conditions. Increased knowledge of sensitisation may lead to the development of new compounds, such as capsaicin based analogues, which can help improve the outcome of such conditions (O'Neill et al. 2012).

1.4.7 PIP_2-induced Desensitisation

Another proposed, yet controversial, method that may induce TRPV1 desensitisation is through PIP_2 hydrolysis (Smutzer & Devassy 2016). PIP_2 can potentiate receptor sensitisation via PLCβ activation (Vriens et al. 2009; Morales-Lázaro et al. 2013). However, PIP_2 cleavage by an isomer of PLC, PLCδ, may potentiate desensitisation (Smutzer & Devassy 2016). The increased intracellular concentration of Ca^{2+}stimulates the activation of PLCδ, which functions the same way as PLCβ to hydrolyse PIP_2 (Vriens et al. 2009; Smutzer & Devassy 2016). Due to the dependence of TRPV1 on PIP_2 to function as an integral membrane protein and its sensitivity to the membrane and phospholipid environment, the depletion of PIP_2 decreases TRPV1 activity and results in desensitisation (Lukacs et al. 2013a; Rohacs 2015; Smutzer & Devassy 2016). This suggests that activation of TRPV1 may be self-limiting. This is so, as second messenger systems that result from TRPV1 activation decrease PIP_2 concentrations within the plasmalemma via PLC hydrolysis, thus regulating TRPV1 activity (Lukacs et al. 2013b). In contrast, *in vitro* activation of TRPV1 by capsaicin continues in the absence of PIP_2 (Cao et al. 2013b; Cao et al. 2013a; Senning et al. 2014).

However, the reintroduction of PIP_2 into the membrane induces subsequent desensitisation of the TRPV1 (Cao et al. 2013b; Cao et al. 2013a; Senning et al. 2014). This suggests that TRVP1 activity may be modulated by PIP_2 depending on what side of the plasmalemma it is found. Hence, further studies are needed to confirm if prolonged activation by capsaicin *in vivo* is a self-limiting process, as this may have consequences for the therapeutic use of capsaicin, in particular, for long-term capsaicin usage in treating obesity and metabolic syndrome.

1.5 Capsaicin as a treatment in pathophysiological disease states

The role of capsaicin in analgesia and TRPV1 physiology has been established. Additionally, TRPV1 expression is abundant throughout the body. Inoue et al. (2006) using reverse transcriptase polymerase chain reaction (RT-PCR) established the distribution of TRPV1 in different cells and tissues, including smooth muscle cells and endothelial cells found in the cardiovascular system. Hence the role of capsaicin in cardiovascular and other disease states will be explored.

1.5.1 Hypertension and renal function

Capsaicin-induced activation of TRPV1 results in the cytosolic Ca^{2+}-induced release of substance P and CGRP, which produces vasodilatation, thus decreasing blood pressure and relieving hypertension (Li & Wang 2003; Fattori et al. 2016). Since TRPV1-containing afferents are intricately associated with the cardiovascular system (Tominaga et al. 1998; Inoue et al. 2006), Li and Wang (2003) imposed a high salt intake onto Wistar rats and noted that oral low-dose capsaicin activated TRPV1 receptors in surrounding cardiovascular tissues. In these rats, CGRP concentrations increased, indicating that capsaicin induced post-synaptic release of CGRP and substance P. Li and Wang (2003) also treated the rats with the TRPV1 antagonist capsazepine and noted that the hypotensive effects of capsaicin were ameliorated. Li and Wang (2008) showed that TRPV1 activation in renal tissue decreased renal pressure, increased glomerular filtration rate and increased water/sodium excretion. Hence, Li and Wang (2008) concluded that TRPV1 activation by capsaicin augments CGRP and substance P release and, therefore, has a key role in mediating renal

function and, consequently, blood pressure. Furthermore, since capsaicin treatment improves glomerular filtration rate and enhances diuresis, it would be expected that creatinine, urate and urea concentrations would be improved in an obese rat model. While it can be appreciated that changes in renal function can indirectly affect blood pressure, more studies are needed to determine the impact capsaicin may convey on renal physiology and treatment of its pathological conditions. However, since renal function is a chronic mediator of blood pressure, future long-term studies would be of benefit to determine the effectiveness of capsaicin as a chronic treatment option (Hall 2015) as it is unknown whether chronic capsaicin administration results in TRPV1 desensitisation within the kidneys and surrounding tissue, making chronic capsaicin an ineffective treatment to regulate renal function and blood pressure.

TRPV1 is expressed by cells of the vascular endothelium (Inoue et al. 2006). Activation of TRPV1 by dietary low-dose capsaicin increased nitric oxide production from endothelial cells (Yang et al. (2010). They postulated that oral low-dose capsaicin would activate endothelial TRPV1, increase cytosolic Ca^{2+}, induce activation of PKA and endothelial nitric oxide synthase (eNOS), resulting in nitric oxide release and subsequent vasodilatation (Yang et al. 2010). Xu et al. (2011) validated this proposal by administering low-dose oral capsaicin to demonstrate increased eNOS concentrations, blood pressure reductions and reduced instances of stroke in hypertensive rats. They indicated TRPV1 knock-out hypertensive mice remained hypertensive and susceptible to stroke in the presence of capsaicin (Xu et al. 2011). Since this study measured cerebrovascular activity, it could be proposed that capsaicin treatment would enhance cognitive function if the cerebral blood flow rate was enhanced. However, further studies confirming this need to be performed. Additionally, further studies are needed to see whether capsaicin crosses the blood-brain barrier and stimulates TRPV1 expressed within the brain, as it remains controversial whether capsaicin crosses the blood-brain barrier (Donnerer et al. 1990; Vermeersch et al. 2015).

Studies in the rat indicate that dietary capsaicin can reach and cross the blood-brain barrier (Donnerer et al. 1990; Hu et al. 2005). Human studies are yet to validate these findings. It is suggested that capsaicin may not cross the blood-brain barrier, but may increase the permeability of the blood-brain barrier by increasing CGRP release in the cerebral cortex (Vermeersch et al. 2015). However, there is consensus that capsaicin can reach the brain via the systemic blood flow (O'Neill et al. 2012). Given that the blood-brain barrier is leaky at the area postrema and median eminence, capsaicin may act centrally by crossing the barrier at this particular cortical region (Bauer et al. 2016). This area is juxtaposed to the hypothalamus, which has an array of functions including the integration and processing of sympathetic and parasympathetic information. Hence, capsaicin may convey its effects centrally to induce changes in satiety, blood pressure, digestion and various psychological behaviours (Hall 2015). Thus, further studies involving vagotomy or vagal ablation in conjunction with capsaicin treatment in rats may assist in validating this hypothesis. Irrespective of the mechanisms involved, it is clear from these studies that TRPV1 plays a pivotal role in hypertension management.

1.5.2 Atherosclerosis and inflammation

Capsaicin may have additional effects on the human cardiovascular system other than the regulation and treatment of hypertension, such as decreasing lipid stores and attenuation of atherosclerotic lesions (Rzucidlo et al. 2007; Ambrosino et al. 2013; Robbins et al. 2013). Atherosclerosis is essentially an inflammatory process with excess lipid accrual in the arterial wall inducing vascular remodelling (Fattori et al. 2016). Vascular remodelling occurs together with vascular smooth muscle cell hypertrophy and hyperplasia in the medial and intimal vascular layers, the migration of these cells from the medial layer to the intimal layer, or both (Rzucidlo et al. 2007; Lacolley et al. 2012; Robbins et al. 2013; McCarty et al. 2015). These changes occur due to vascular smooth muscle cell phenotypic switching between three phenotypes, synthetic, macrophage-like and osteochondrogenic, in response to stimuli including proinflammatory mediators and reactive species (Ambrosino et al. 2013; Salabei & Hill 2015).

Li et al. (2014) reported that foam cell formation is the primary contributing factor of atherosclerosis. Foam cells were thought to be macrophages, however new evidence indicates that the majority of foam cells are vascular smooth muscle cells exhibiting a macrophage-like phenotype (Lacolley et al. 2012; Li et al. 2014; Salabei & Hill 2015). Foam cells accumulate cytoplasmic droplets of cholesterol esters and triglycerides, as they express different cholesterol uptake receptors and reverse cholesterol transporters, such as low-density lipoprotein (LDL) receptor (LDLR), LDLR related protein 1 (LRP1) and ATP-binding cassette transporter A1, or ABCA1 (Ma et al. 2011; Li et al. 2014; Fattori et al. 2016). Furthermore, *in vitro* TRPV1 activation by capsaicin in these cells increased cytosolic Ca^{2+}, reduced lipid accumulation, decreased LRP1 expression and increased ABCA1, which all enhance cellular cholesterol efflux and reduce cholesterol uptake (Ma et al. 2011). Ma et al. (2011) successfully replicated and validated these *in vitro* findings in mice treated with 0.01% capsaicin administered orally in the presence of a high-fat diet. Therefore, since increased cytosolic Ca^{2+} activates PKA-mediated pathways and calcineurin, further studies are needed to determine if these factors act in concert, independently or if another intracellular mechanism is stimulated upon capsaicin-mediated TRPV1 activation. Further studies using capsaicin are needed to determine its role in inhibiting foam cell development and, consequently, atherosclerosis.

Li et al. (2014) demonstrated that 0.01% dietary capsaicin relieved atherosclerosis by inhibiting foam cell formation and by inducing autophagy in oxidised LDL treated vascular smooth muscle cells. Autophagy is a self-degrading homeostatic mechanism that dismantles and recycles organelles and nutrients such as lipids (Li et al. 2014; Salabei & Hill 2015). Triggering TRPV1 lowered proinflammatory mediator concentrations which led to decreased foam cell formation (Li et al. (2014). This project also established that capsaicin stimulated TRPV1 and induced autophagy through an adenosine monophosphate activated protein kinase (AMPK) signalling mechanism, which in the presence of reactive species such as oxidised LDL is suppressed and results in foam

cell formation. Hence, Li et al. (2014) concluded that foam cell migration was decreased, whilst autophagy was enhanced when TRPV1 was triggered by low-dose dietary capsaicin.

Kiyan et al. (2014) validated that atherosclerosis results from decreased vascular smooth muscle cells autophagy, enhanced inflammation and subsequent migration of vascular smooth muscle cells and differentiation leading to foam cell formation. Wei et al. (2013) confirmed the importance of TRPV1 in regulating atherosclerosis by demonstrating TRPV1 knockout mice fed a high fat diet showed increased concentrations of cholesterol, cytokines and chemokines, which promoted formation of atherosclerotic plaques. Therefore, targeting TRPV1 with capsaicin could alleviate atherosclerosis and the processes that induce the condition. Furthermore, since atherosclerosis and hypertension increase the probability of stenosis, myocardial infarction, stroke, angina, thrombosis and embolism, capsaicin treatment could decrease the probability of these events. However, selected studies should determine if TRPV1 is an appropriate target in treating these secondary disease states. Additionally, future studies could consider capsaicin usage in treating thrombotic episodes, as this may have consequences on platelet aggregation, bleeding disorders and blood viscosity control. Furthermore, given that vascular smooth muscle cells and gastrointestinal smooth muscle cells are closely related and both possess the ability to undergo hypertrophy and hyperplasia, the mechanisms outlined above may be transferrable to the gastrointestinal smooth muscle cells (Blennerhassett et al. 1992; Hall 2015). Cells of the gastrointestinal tract also express LRP1 and ABCA1, thus indicating a potential role for these cells in lipid metabolism (Liu et al. 2011; Tarling et al. 2013). Additionally, given that TRPV1-expressing afferents innervate the gastrointestinal tract, capsaicin administration may change gastrointestinal cell lipid metabolism and inflammatory bowel disorders via similar mechanisms (Inoue et al. 2006). Before these hypotheses can be tested, future investigations need to initially identify whether gastrointestinal smooth muscle cells possess the ability to phenotypically switch in the presence of inflammation in a manner similar to vascular smooth muscle cells.

1.5.3 Hepatic steatosis, obesity and metabolic dysfunction

The chronic release of free fatty acids and proinflammatory chemokines by adipocytes induces hepatic dysfunction by causing chronic inflammation, hepatocyte apoptosis, enhanced fat deposition and hepatic insulin resistance (Kang et al. 2010; Wei et al. 2013; McCarty et al. 2015). This ultimately results in hepatic steatohepatitis as well as other metabolic disease states such as type 2 diabetes, insulin resistance, dyslipidaemia and cardiovascular disease (Kang et al. 2010; Wei et al. 2013; McCarty et al. 2015). Dietary low-dose capsaicin administered to mice and rats reduced systemic concentrations of interleukin-6 (IL6), tumour necrosis factor-α (TNFα), monocyte chemoattractant protein-1 (MCP1), macrophage inflammatory protein-2 (MIP2) and cyclooxygenase-2 (COX2), as mediators of obesity-induced inflammation (Kang et al. 2010; Wei et al. 2013; Borbély et al. 2015; McCarty et al. 2015).

TRPV1 knockout mice had increased systemic concentrations of these factors, whilst mice expressing TRPV1 exhibited decreased concentrations when treated with a TRPV1 agonist, such as capsaicin (Wei et al. (2013). These factors were decreased in adipocytes when dietary low-dose capsaicin was administered orally in mice, together with reduced plasma concentrations of glucose, insulin and triglycerides (Kang et al. (2010). Wei et al. (2013) supported these findings, but additionally reported decreased LDL concentrations in TRPV1 active mice. These findings suggest that capsaicin upregulates LDLR and ABCA1, which promote cholesterol clearance, thus reducing dyslipidaemia (Kang et al. 2010; Ma et al. 2011; Wei et al. 2013; Li et al. 2014). Furthermore, this demonstrates that these inflammatory mediators block insulin receptors, thus causing glucose intolerance and contributing to dyslipidaemia (Kang et al. 2010; Wei et al. 2013; Borbély et al. 2015). Capsaicin-induced TRPV1 activation alleviates this by diminishing their production, thus restoring glucose and lipid homeostasis (Kang et al. 2010; Wei et al. 2013). While it can be postulated that these effects occur in a similar mechanism, as outlined in vascular smooth muscle cell dysfunction, it does not account for findings such as increases in adiponectin and peroxisome proliferator-activated receptor gamma (PPARγ), which both function to assist in regulating glucose

and lipid metabolism (Kang et al. 2010; Ambrosino et al. 2013; Wei et al. 2013). Therefore, more studies into the mechanisms by which capsaicin alleviates these disorders need to be performed. However, given that these factors alleviate these symptoms, it would be expected that plasma cholesterol, triglycerides, glucose and insulin concentrations would decline and that liver function markers, such as bilirubin, would also decline and return to normal in an obese rat treated with capsaicin.

Wang et al. (2012) demonstrated the effect of dietary capsaicin administered orally in ameliorating glucose and lipid dysregulation. They noted increased concentrations of glucagon-like peptide-1 (GLP1) in mice fed a high fat diet concurrent with a return of glucose and lipid homeostasis when treated with capsaicin. GLP1 is a gastrointestinal hormone released by ileac L cells (Wang et al. 2012). GLP1 decreased glucagon expression and upregulated insulin release (Wang et al. 2012; McCarty et al. 2015). Wang et al. (2012) suggested that GLP1 was released in response to Ca^{2+} influx. Since GLP1 secretion is in response to increased plasma glucose, the stimulation of GLP1 on the pancreas via GPCR, which potentiates TRPV1 activation, exacerbates the effects of glucagon downregulation and enhances the effects of insulin via the mechanisms listed previously. However, the exact mechanisms by which TRPV1 stimulation enhances GLP1 release have not been elucidated and provide a promising direct target for type 2 diabetes treatment. Furthermore, since vagal afferents supplying the gastrointestinal tract contain TRPV1, capsaicin may activate the gut-brain axis and induce central GLP1 release. Therefore, capsaicin may not only elicit a GLP1 response in regulating glucose homeostasis, it may also elicit GLP1-induced satiation. This hypothesis may also be related to other satiety-related hormones such as peptide YY_{3-36}, cholecystokinin, leptin, ghrelin and serotonin, given that vagal afferents act as an information highway linking the gut and the brain (Prechtl & Powley 1990; Berthoud et al. 1995; Bauer et al. 2016). Hence, further studies are needed in both animal and human models to determine the effects capsaicin may convey in satiation, energy intake and, therefore, energy homeostasis.

Brown adipose tissue (BAT) is activated by capsaicin ingestion (Kawabata et al. 2009; Ono et al. 2011). These authors suggest that TRPV1 activation by capsaicin in afferents supplying the gastrointestinal tract stimulate vagal nerve activity, which consequently triggers selective sympathetic neurons supplying BAT (Kawabata et al. 2009; Ono et al. 2011). Ludy et al. (2011) suggest that there is an interaction between sympathetic nervous system activity and food consumption whereby when this activity increases, consumption declines. Hence, enhanced sympathetic activity may increase satiety, thus reducing energy consumption, and increase BAT stimulation which, as demonstrated by Ono et al. (2011) and Kawabata et al. (2009), increases thermogenesis metabolism and energy expenditure. Janssens et al. (2014) demonstrated increased satiety, energy expenditure and hypothalamic activity in human trials with capsaicin, which supports the notion. However, since GLP1 concentrations increase upon capsaicin ingestion, it cannot be excluded from inducing satiety by indirectly regulating ghrelin and leptin concentrations, thus relieving hunger pains (Diepvens et al. 2007; Ludy et al. 2011). Additionally, these two pathways may act together. Although the mechanism has not been fully elucidated, it can be appreciated that these factors are important to study in relation to metabolic dysfunction and obesity.

1.6 The animal model used for determining the effects of capsaicin

The published literature identifies the complications associated with obesity and metabolic syndrome, using a range of animal models to study these pathophysiological changes and the potential capsaicin conveys in treating these changes. This project aims to use capsaicin in attenuating diet-induced metabolic syndrome. Hence, a valid animal model mimicking the pathophysiological changes associated with metabolic syndrome is required to identify treatment options in humans (Panchal & Brown 2011). Additionally, since obesity is the chief predisposing factor associated with its development, a diet incorporating the features of a modern westernised diet is required. The western diet is referred to as hypercaloric and highly palatable, due to large

quantities of simple sugars and carbohydrates, such as fructose, and saturated and *trans* fats, typically in the form of animal fats (McAllister et al. 2009; Sadowska et al. 2017). This diet has been linked to overeating and, therefore, contributes to the excessive energy intake that partly defines obesity (Sadowska et al. 2017).

A model that incorporates a high-carbohydrate, high-fat diet comprised of fructose, condensed milk and beef tallow for its macronutrient and micronutrient composition and the response that this induces in male Wistar rats has been developed and used in a variety of studies initiated by the Functional Foods Research Group at the University of Southern Queensland (Panchal & Brown 2011; Panchal et al. 2012; Diwan et al. 2013; Panchal et al. 2013). These rats developed metabolic complications, cardiovascular changes, hepatic dysfunction, increased organ wet weights and systemic inflammation. These studies have used a low-glycaemic cornstarch diet as a control diet, as it does not typically elicit any metabolic, structural or functional changes in male Wistar rats. However, it is important to note that rats do not appear to develop atherosclerosis and do not exhibit a notable change in plasma HDL and LDL concentrations (Chen et al. 2014). Hence, future studies should explore that possibility of translating this model into other animals, such as mice, which develop atherosclerosis and pronounced changes in plasma HDL and LDL concentrations (Ishibashi et al. 1994). However, this particular animal model provides a valid method to investigate the potential reversal effects that different therapeutic options, such as nutraceuticals may have on metabolic syndrome. Thus far, the Queen Garnet plum, coffee extract, olive leaf extract, cardamom, piperine and purple carrot juice have elicited favourable health benefits and are, therefore, identified as potential therapeutic agents in the treatment of the pathophysiological states associated with obesity and metabolic syndrome (Poudyal et al. 2010b; Poudyal et al. 2010a; Panchal et al. 2012; Diwan et al. 2013; Panchal et al. 2013; Bhaswant et al. 2015b; Bhaswant et al. 2015a).

The model used in these studies is 16 weeks in length and all rats are either fed the high-carbohydrate, high-fat diet or cornstarch diet. An intervention is introduced to experimental high-

carbohydrate, high-fat diet and cornstarch diet groups at 8 weeks for a further 8 weeks to assess its efficacy in attenuating metabolic syndrome. However, it is important to note that this protocol does not assess the long-term efficacy and adverse effects of a treatment. Hence, future studies using this model should aim to extend the treatment period to assess the efficacy and adverse effects that a treatment may convey over an extended period. Nonetheless, this study will follow the same timeframe as outlined in these studies, as it provides a foundation to assess the potential efficacy capsaicin may convey in attenuating metabolic syndrome.

1.7 Aim and hypotheses

The published literature shows that low-dose dietary capsaicin may attenuate the individual changes in metabolic syndrome. However, this will be the first study to assess the efficacy of capsaicin in treating the range of changes seen in diet-induced metabolic syndrome. Therefore, the aim of this study is to observe whether low-dose dietary capsaicin (5mg/kg bodyweight/day) attenuates the pathophysiological changes of metabolic syndrome in an obese rat model. Subsequently, this will support studies to translate these findings to humans by undertaking human clinical trials. In particular, this study will determine whether low-dose dietary capsaicin can be used as an inexpensive and novel therapeutic agent to attenuate:

- Biomarkers of obesity, which include fat mass, abdominal circumference, body mass index (BMI) and systemic tissue inflammation;
- Impaired cardiovascular structure and function, defined as hypertension, increased diastolic stiffness within the heart, increased collagen deposition and myocardial wet weight;
- Reduced metabolic function, defined as glucose intolerance, dyslipidaemia and an increased energy intake; and
- Non-alcoholic fatty liver disease, defined by increased fat vacuole deposition, inflammatory cell infiltration, liver wet weight and plasma activities of liver function enzymes.

It is hypothesised that:

- Low-dose dietary capsaicin will not alter physiological responses in rats fed the cornstarch diet.
- Low-dose dietary capsaicin will attenuate the cardiovascular, liver, metabolic and inflammatory changes in rats fed a high-carbohydrate, high-fat diet.

Chapter 2: Methods and materials

2.1 Materials

Chillies, usually from lal mirch and cayenne cultivars belonging to *Capsicum annum* species, were purchased from the local Indian grocery store at the Uni Plaza Shopping Centre, Darling Heights, QLD, Australia. Beef tallow was purchased from Carey Brothers, Warwick, QLD, Australia. Condensed milk was purchased from Coles Kearney Springs, Toowoomba, QLD, Australia. Meat-free powdered rat food was purchased from Speciality Feeds, Glen Forrest, WA, Australia. Hubble, Mendel and Wakeman salt mixture was purchased from MP Biomedicals, Seven Hills, NSW, Australia. Fructose was purchased from Tate & Lyle, Wacol, QLD, Australia. Corn starch was purchased from Agri Food Ingredients, Kew East, VIC, Australia. All laboratory chemicals were purchased from Sigma-Aldrich Australia, Castle Hill, NSW, Australia, unless otherwise specified at their first appearance in text.

2.2 Capsaicin extraction

Initially, 740 g of chillies were dried at 65°C in a fan-forced oven for 24 hours. Chilli seeds were removed and chillies were subsequently crushed into smaller pieces using a domestic Kenwood food processor. Approximately 90 g of the chilli flakes were placed into the extraction shell of a Soxhlet extractor (B34). Analytical grade methanol (350 mL, Lab-Scan Analytical Sciences, Bangkok, Thailand) was placed into a round-bottom volumetric flask suspended in 500 mL of synthetic ultra-grade vacuum-pump oil (Edwards, Mississauga, Ontario, Canada), which was heated to 240°C by a Velp Scientific ARE heating and magnetic stirrer (Rowe Scientific, Perth, WA, Australia) to induce the methanol to percolate (boil and reflux) through the sample (Ashwini et al. 2015b). The time of each cycle (boil and reflux) was measured for each run of 100 g of chillies. At least 15-20 cycles per run were performed as previously described (Goci et al. 2014; Ashwini et al. 2015b). Eight Soxhlet extraction runs were performed in total with approximately 90 g of chillies used per run.

Upon completion of the Soxhlet extraction process, the contents of the round-bottom volumetric flask (methanol containing extract) were removed and placed on to a Laborota 4000 rotary evaporator to evaporate the methanol from the flask (Betts 1999). The contents of the flask were mixed with 100mL of analytical grade dichloromethane (Honeywell Burdic and Jackson, Muskegon, MI, USA), in which the capsaicin is readily soluble (Wagner & Bladt 1996; Oliveira et al. 2004). This mixture along with 100 mL of water was placed into a 1L separatory funnel. The water was added to remove any water-soluble products that may have been extracted from the chillies (Ashwini et al. 2015b). The contents of the flask were mixed and the water and dichloromethane mixtures were separated until the dichloromethane mixture was clean and free of water.

Sodium hydroxide solution (0.25 M; 5g of anhydrous NaOH pearls/500mL of distilled water; Sigma-Aldrich Australia, Sydney, NSW, Australia) was prepared and 100 mL of the solution was placed with the dichloromethane layer into the 1L separatory funnel. The sodium hydroxide was added to remove any oil, fat and mineral products that may have been extracted from the chillies and that are readily soluble in its presence (Wagner et al. 2011). The contents of the flask were mixed and the sodium hydroxide and dichloromethane layers were separated until the dichloromethane mixture was clean and free of sodium hydroxide. 8 mL of analytical grade 32% hydrochloric acid (BIOLAB, Clayton, Vic, Australia) was added to the sodium hydroxide layer to neutralise the sample and this was mixed with the dichloromethane layer in the 1L separatory funnel. The neutralised and dichloromethane layers were mixed and separated out until the dichloromethane mixture was clean and free of the neutralised blend (Wagner et al. 2011; Ashwini et al. 2015b). The final dichloromethane layer was decanted into a pre-measured round-bottom flask and this flask was placed onto the rotary evaporator to evaporate the dichloromethane from its contents. The residual product in the round-bottom flask and the flask itself were measured to determine the quantity of capsaicin extracted. The product was then transferred to a pre-measured glass vial and the vial and its contents were measured to verify the quantity of capsaicin extracted.

This vial was kept under locked storage as per instructions outlined in its Material Safety Data Sheet.

The presence of capsaicin was demonstrated by proton nuclear magnetic resonance spectroscopy (^{1}H-NMR). This procedure was performed by Dr Robert Reid at the Institute of Molecular Bioscience, The University of Queensland. A 5 mg sample of the extract was dissolved in 0.5-1 mL of deuterated chloroform and placed into a ^{1}H-NMR tube. ^{1}H-NMR spectra were recorded at 600.13MHz using a Bruker Avance spectrometer (Karlsruhe, Germany). Eight scans of 32K data points were attained at 298K using a spectral width of 10303Hz (10ppm), acquisition time of 2.28 seconds, relaxation delay of 20 seconds, flip-angle of 90° and pulse duration of 20 microseconds (Reid et al. 1996; Nazari et al. 2007).

An approximate percentage of the compounds isolated in the extract was calculated. This was performed by identifying recognisable peaks in the ^{1}H-NMR of pure capsaicin and dihydrocapsaicin that occur at different chemical shifts and the relative integrated peak intensities were compared (Wagner et al. 2011). More specifically, the doublet of the isopropyl group of capsaicin and dihydrocapsaicin, which occurs approximately between 1.00 parts per million (ppm) and 0.80 ppm, were used to approximate the relative quantities of the obtained extract (Yao et al. 1994; Wagner et al. 2011). The recognisable peak areas for capsaicin and dihydrocapsaicin were matched against their respective standard curve to estimate their approximate percentages within the extract. The approximate percentage of capsaicin (% capsaicin) was determined by [(area of capsaicin)/(area of capsaicin + areas of unknown)]x100 (Yao et al. 1994; Wagner et al. 2011).

2.3 Rats, experimental groups and housing

All experimental protocols were approved by the Animal Ethics Committee of the University of Southern Queensland (project number – 15REA006, valid from 22/08/2016 until the 22/10/2018) under the guidelines of the National Health and Medical Research Council of Australia. Male Wistar rats (8-9 weeks old; 338 ± 7 g, n = 47) were purchased from the Animal Resource Centre,

Murdoch, WA, Australia. Rats arrived three weeks prior to commencing their diets and were acclimatised for three weeks on laboratory chow diet. One rat was found to possess a malocclusion and was euthanased by the University of Southern Queensland Animal Welfare Officer. The rats were randomly divided into four experimental groups: corn starch diet-fed rats (C; $n = 11$), corn starch diet-fed rats supplemented with capsaicin (CC; 0.015% of diet; $n = 12$); high-carbohydrate, high-fat diet-fed rats (H; $n = 12$) and high-carbohydrate, high-fat diet-fed rats supplemented with capsaicin (HC; 0.015% of diet; $n = 12$).

C and H rats were fed with cornstarch and high-carbohydrate, high-fat diets, respectively, for 16 weeks. CC and HC rats were also fed with cornstarch and high-carbohydrate, high-fat diets, respectively, for 16 weeks with the diets supplemented with capsaicin (0.015% of diet) for the last 8 weeks of the protocol. The cornstarch and high-carbohydrate, high-fat diets have been previously described in detail, and are included in Table 3 (Panchal et al. 2011). Drinking water for H and HC rats was supplemented with 25% fructose, whereas C and CC rats were given drinking water without any additive. All rats were individually housed in temperature-controlled (21 ± 2ºC) 12-hour light/dark cycle conditions within the animal house at the University of Southern Queensland, Toowoomba campus and were given *ad libitum* access to diet and water.

Table 3: Composition of diet

Components (g/kg)	C	H
Corn starch	570g	-
Fructose	-	175g
Condensed milk	-	395g
Beef tallow	-	200g
Salt mixture	25g	25g
Powdered rat food	155g	155g
Water	250mL	50mL
Drinking water	No additives	25% fructose (w/v)

Reference: (Panchal et al. 2011) – modified.

2.4 Daily measurements

Daily measurements of body weight and food and water intake were taken, which permitted the monitoring of the day-to-day health of the rats. The bedding of the rats was assessed on a regular

basis and changed when required to ensure a clean and hygienic environment was maintained. Energy densities of the C and H diets were used to calculate energy consumption of the rats and feed conversion efficiency (%) was calculated as [mean body weight gain (g)/daily energy intake (kJ)] x 100 (Novelli et al. 2010).

2.5 Systolic blood pressure

Systolic blood pressure of rats was measured at 0, 8 and 16 weeks under light anaesthesia with Zoletil (tiletamine 10 mg/kg, zolazepam 10 mg/kg, intraperitoneal; Virbac, Peakhurst, NSW, Australia), using an MLT1010 Piezo-Electric Pulse Transducer (ADInstruments, Sydney, Australia) and inflatable tail-cuff connected to an MLT844 Physiological Pressure Transducer (ADInstruments, Sydney, Australia) and PowerLab data acquisition unit (ADInstruments, Sydney, Australia) (Panchal et al. 2011). A pressure meter was used to calibrate the transducer on the day of measurement and was conducted as per manufacturer's instructions. Room temperature was set to 25ºC prior to the sedation procedure. Once sedated, the tails of the rats were inserted through the tail cuff and the transducer was wrapped around the tail alongside the cuff. Five to six individual systolic blood pressure readings were recorded for each rat and the mean was calculated for each rat prior to using the measurement for group recordings. 5mL of saline was administered subcutaneously following the systolic blood pressure measurements to avoid dehydration. Prior to the rats being returned to their individual housing, recovery of the rats from anaesthesia was monitored every fifteen minutes for the first hour and then once every hour until complete recovery was ascertained.

2.6 Body composition measurements, abdominal circumference and body length

Body composition analyses of rats were measured at 8 and 16 weeks under light anaesthesia using Zoletil (tiletamine 10 mg/kg, zolazepam 10 mg/kg, intraperitoneal; Virbac, Peakhurst, NSW, Australia). Room temperature was set to 25ºC prior to the sedation procedure commencing. Body composition analyses were measured using a Norland XR-46 Dual-energy X-ray Absorptiometry

(DXA) densitometer (Norland Corp., Fort Atkinson, WI, USA). The data was analysed using the manufacturer's recommended software for use in laboratory animals (Small Subject Analysis Software, version 2.5.3/1.3.1; Norland Corp.). 10 mL of saline was administered subcutaneously following the DXA measurements to avoid dehydration. Prior to the rats being returned to their individual housing, the recovery of the rats from anaesthesia was monitored every fifteen minutes for the first hour and then once every hour until complete recovery was ascertained. Abdominal circumference was measured at 0, 8 and 16 weeks in conjunction with body composition measurements. A standard measuring tape was utilised to measure abdominal circumference when the rats were placed in the ventral recumbent position (Panchal et al. 2011). Additionally, body length (nose to anus) was measured at the end of the 16 week protocol to calculate body mass index (BMI), determined by body weight (in grams)/[body length (in centimetres)^2] (Novelli et al. 2010).

2.7 Oral glucose tolerance test

At 0, 8 and 16 weeks of the protocol, rats were deprived of all diet types for 12 hours (6pm to 6am). During the fasting period, fructose-supplemented drinking water in the H and HC groups was replaced with normal drinking water. Oral glucose tolerance tests (OGTT) were performed after determining overnight fasting blood glucose concentrations in tail vein blood using Medisense Precision Q.I.D. glucose metres (Abbott Laboratories, Belford, MA). A drop of blood from the tail of the rats was obtained via a prick using a 30 gauge needle, which was subsequently transferred onto a glucose strip (Freestyle Optimum Blood Glucose Test Strips, Abbott Diabetes Care Ltd., Witney, Oxon, UK) attached to the glucometer. Following this, rats were administered a glucose load of 2 g/kg body weight as 40% glucose solution via oral gavage and blood glucose concentrations were measured again at 30, 60, 90 and 120 minutes post oral glucose administration (Panchal et al. 2011). The area under the curve was calculated by plotting blood glucose concentrations against time over the 120 minute period. Rats recommenced their specific diets following completion of the OGTT.

2.8 Euthanasia and isolated Langendorff heart preparation

Rats were euthanased with Lethabarb (pentobarbitone sodium, 100 mg/kg, intraperitoneal; Virbac, Peakhurst, NSW, Australia). After euthanasia, heparin (200 IU) was injected through the right femoral vein. The abdomen was opened and blood (~5 mL) withdrawn from the abdominal aorta, collected into heparinised tubes and centrifuged at 5000 x g for 15 minutes to obtain plasma. Plasma was stored at -20ºC for further biochemical analysis. Hearts were removed and used in isolated Langendorff heart preparations to assess left ventricular function of the rats. Hearts isolated from rats were perfused with modified Krebs-Henseleit bicarbonate buffer containing (in mmol/L): NaCl, 119.1; KCl, 4.75; $MgSO_4$, 1.19; KH_2PO_4, 1.19; $NaHCO_3$, 25.0; glucose, 11.0; and $CaCl_2$, 2.16. Buffer was bubbled with 95% O_2–5% CO_2 and maintained at 35ºC. Isovolumetric ventricular function was measured by inserting a latex balloon catheter into the left ventricle connected to a Capto SP844 MLT844 physiological pressure transducer and Chart software on a Maclab system. Left ventricular end-diastolic pressure values were measured during pacing of the heart at 250 beats/minute using an electrical stimulator. End-diastolic pressures were obtained from 0 to 30 mmHg for calculation of diastolic stiffness constant (κ, dimensionless) (Panchal et al. 2011).

2.9 Organ weights

Upon completion of cardiac perfusion studies, hearts (n = 10 from each group) were divided into right ventricle and left ventricle (with septum) and weighed. Livers (n = 8-12 from each group) were removed and weighed. Retroperitoneal, epididymal and omental abdominal fat pads were removed individually and weighed. These organ weights were normalised against the tibial length (50.5 ± 0.8mm) at the time of organ removal and expressed as mg/mm of tibial length (Panchal et al. 2011). The weights of the various fat pads were substituted into the following formula to determine visceral adiposity index (%): ([retroperitoneal fat (g) + omental fat (g) + epididymal fat (g)]/[body weight (g)]) x 100 (Panchal et al. 2011).

2.10 Thoracic aorta organ bath

Once the heart was removed and blood collected for biochemical analyses, the thoracic aorta was removed and excess adipose tissue was removed from the thoracic aorta to expose the underlying tissue. Thoracic aortic rings (~4 mm in length) were suspended in an organ bath filled with Tyrode physiological salt solution bubbled with 95% O_2–5% CO_2 maintained at 35ºC and the rings were allowed to stabilise at a resting tension of ~10 mN. The Tyrode physiological salt solution contains (in mmol/L): NaCl, 136.9; KCl, 5.4; $CaCl_2$, 1.8; $MgCl_2.H_2O$, 1.05; $NaH_2PO_4.H_2O$, 0.42; $NaHCO_3$, 22.6; ethylenediamine-tetraacetic acid (EDTA), 0.05; ascorbic acid, 0.3; glucose, 5.5. Cumulative concentration response curves (contraction) were obtained for noradrenaline (Sigma-Aldrich Australia, Sydney, NSW, Australia) and cumulative concentration-response curves (relaxation) were obtained for acetylcholine (Sigma-Aldrich Australia) and sodium nitroprusside (Sigma-Aldrich Australia) following submaximal (~70%) contraction to noradrenaline (Panchal et al. 2011).

2.11 Ileum and colon organ bath

Approximately 1-2 cm long distal ileum and distal colon pieces were suspended in an organ bath filled with Tyrode physiological salt solution bubbled with 95% O_2–5% CO_2 and maintained at 35ºC and allowed to stabilise at a resting tension of ~10 mN. Concentration response curves were obtained for acetylcholine by measuring responses to individual concentrations of acetylcholine.

2.12 Tissue histology

Two rats per group were taken exclusively for histological analysis. Two slides were prepared per tissue specimen and two random, non-overlapping fields per slide were taken to avoid biased analysis. Organs were collected from rats used for Langendorff perfusion studies.

Immediately after removal, heart and liver were fixed in 10% neutral buffered formalin and then dehydrated and embedded in paraffin wax. Thin sections (5μm) of tissues were cut and stained with

haematoxylin and eosin stain for determination of inflammatory cell infiltration and fat vacuole enlargement with 20× objective (Panchal et al. 2011). Collagen distribution was defined in the heart with picrosirius red stain. Laser confocal microscopy (Zeiss LSM 510 upright Confocal Microscope) with colour intensity quantitated using the National Institutes of Health (NIH) Image software (NIH, Bethesda, MD, USA) was used to determine the extent of collagen deposition in tissue sections (Panchal et al. 2011).

2.13 Biochemical analysis

Plasma activities of alanine transaminase (ALT) and aspartate aminotransferase (AST), and plasma concentrations of total cholesterol, triglycerides, non-esterified fatty acids (NEFA) were determined as previously described (Panchal et al. 2011). These variables were measured using kits and controls supplied by Olympus (Tokyo, Japan) using an AU 400 Olympus analyser at the School of Veterinary Sciences, the University of Queensland, Gatton campus. NEFA in plasma was determined using a commercial kit (Wako Diagnostics, Osaka, Japan) and plasma concentrations of insulin were measured using commercial kits according to manufacturer-provided standards and protocols at the School of Veterinary Sciences, The University of Queensland, Gatton campus (Panchal et al. 2011).

2.14 Statistical analysis

All data are presented as mean ± standard error of the mean (SEM). Results were tested for variance using Bartlett's test and variables that were not normally distributed were transformed (using log 10 function) prior to statistical analyses. All the groups were tested for effects of diet, treatment and their interaction by two-way analysis of variance. When the interactions and/or the main effects were significant, then the groups were compared using the Newman-Keuls multiple comparison post-test. A $P<0.05$ was considered statistically significant. All statistical analyses were performed using Prism version 6.00 for Windows (Graphpad Software, San Diego, CA, USA).

Chapter 3: Results

3.1 Capsaicin extraction and purification

Crude extract of capsaicin from dried chillies from cultivars of *Capsicum annum* species was obtained by using methanol as a solvent to percolate through a Soxhlet extractor. A summary of this procedure is given in Table 4. The crude extract was then subjected to a rigorous cleaning process to purify the capsaicin extract, as outlined in section 2.2 capsaicin extraction. The final product was a dark red oily extract. The final weight of extract is given in Table 5. Finally, the presence of capsaicin was determined by ^{1}H-NMR spectroscopy, which provided structural evidence about the compounds obtained by extraction following chemical treatment and clean-up (Figure 6A). The presence of dihydrocapsaicin and remnant dichloromethane as the solvent was also determined. The protons producing the different spectral peaks are defined as follows:

- Capsaicin – 1.10-1.20ppm = 14- and 15-CH_3; 1.70-2.30ppm = 11-, 12- and 13-CH_2; 2.70-2.80ppm = 2 unrelated groups 9,10; 3.70ppm = 8-OCH_3; 4.30ppm = 7- benzylic CH_2; 5.30-5.40ppm = 5,6 two doublets (double bond); 5.70ppm = NH; 5.80ppm = 4- aromatic OH; 6.80ppm = 1-3 aromatic protons;
- Dihydrocapsaicin – 0.80-0.90ppm = 14- and 15-CH_3; 1.80ppm-2.3ppm = 9-13 CH_2; 2.70ppm = 8-OCH_3; 3.9ppm = 7- benzylic CH_2; 4.10-4.30ppm = 2 unrelated groups, 5,6; 5.70ppm = NH; 5.80ppm = 4- aromatic OH; 6.80ppm = 1-3 aromatic protons; and
- Dichloromethane – 5.30ppm, single peak.

An approximate percentage of the compounds isolated in the extract were calculated (Table 5). This was performed by identifying recognisable peaks in the ^{1}H-NMR of capsaicin and dihydrocapsaicin that occur at different chemical shifts and the relative integrated peak intensities were compared (Figure 6B).

Table 4: The number of cycles and cycle times per Soxhlet extraction run to obtain crude capsaicin extracts from dried chillies.

Run number	Dried chilli quantity (g)	Start time (24hr)	Finish time (24hr)	Run time (hrs)	Number of cycles (boil and reflux)	Average cycle time (min)
1	120	10:40:00	19:45:00	9:05	21	26
2	65	14:33:00	07:10:00	16:37	20	21
3	100	12:05:00	06:35:00	18:30	25	44
4	60	17:17:00	06:50:00	13:33	24	33
5	95	10:25:00	19:50:00	9:25	17	33
6	110	12:20:00	11:00:00	22:40	24	55
7	90	10:20:00	19:25:00	9:05	23	23
8	100	13:15:00	11:00:00	21:45	21	60
Average	93	12:37:00	12:42:00	15:08	22	37
SEM	6	N/A	N/A	3	1	5
N	8	8	8	8	8	8

Values are mean ± SEM, *n*=8.

Table 5: The weight of capsaicin obtained per clean-up round and the final yield and weight of capsaicin obtained

Run Number	Clean-up round	Vial weight (g)	Vial weight + capsaicin (g)	Capsaicin obtained (g)
1 **2**	1	84.50	85.30	0.80
3 **4** **5**	2	91.14	93.02	1.88
6 **7**	3	86.63	93.38	6.75
8	4	86.73	87.79	1.06
			Total capsaicin obtained (g)	10.49
			Total capsaicin yield (%)	1.42
			Approximate capsaicin ratio determined by ^{1}H-NMR (%)	86
			Approximate dihydrocapsaicin ratio determined by ^{1}H-NMR (%)	14

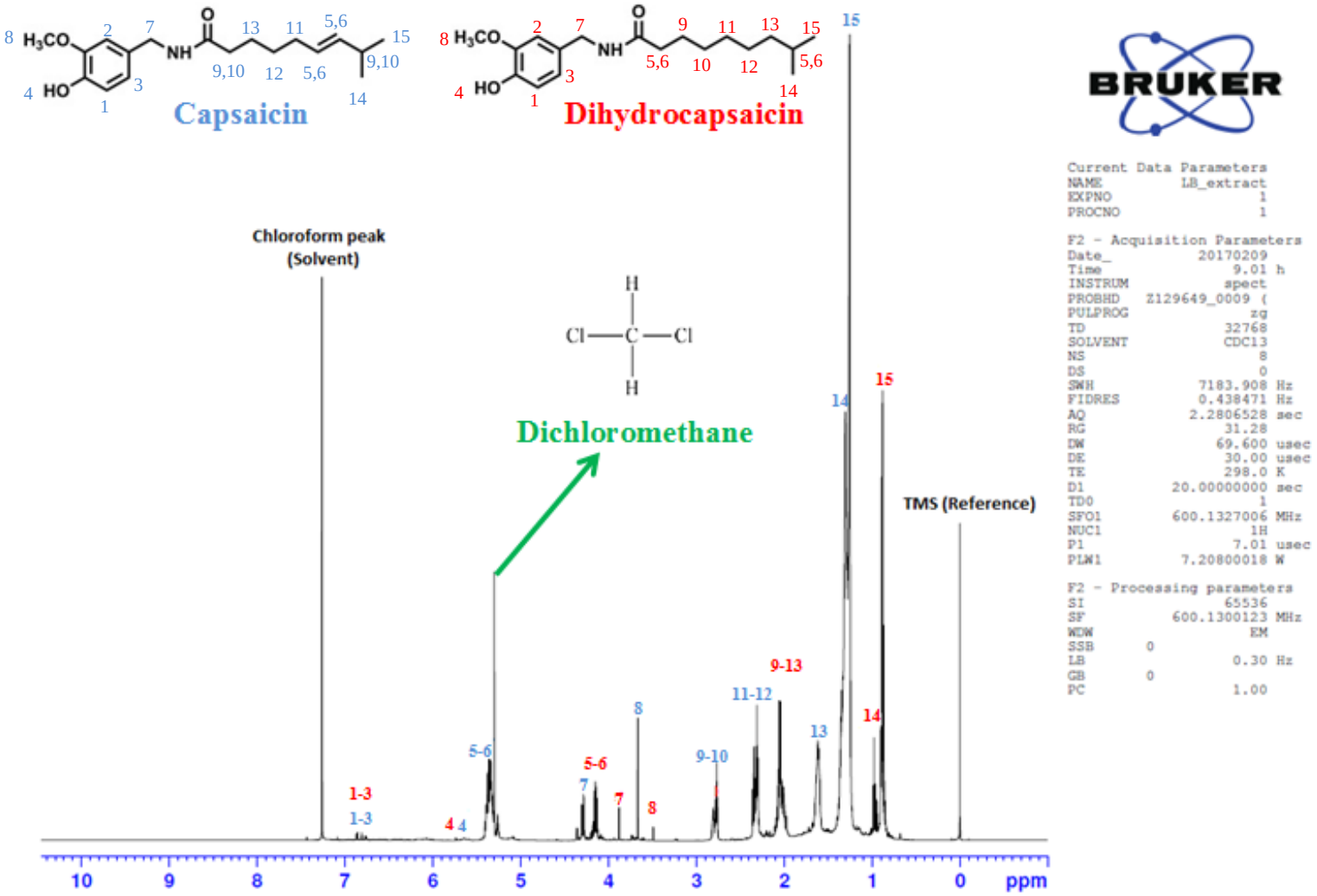


Figure 6a: The structural identification of capsaicin by 1H-NMR. A 5mg sample of the extract was dissolved in 0.5-1 mL of deuterated chloroform and placed into a 1H-NMR tube. 1H-NMR spectra were recorded at 600.13MHz using a Bruker Avance spectrometer. Eight scans of 32K data points were attained at 298K using a spectral width of 10303Hz (10 ppm), acquisition time of 2.28 seconds, relaxation delay of 20 seconds, flip-angle of 90° and pulse duration of 20 microseconds.

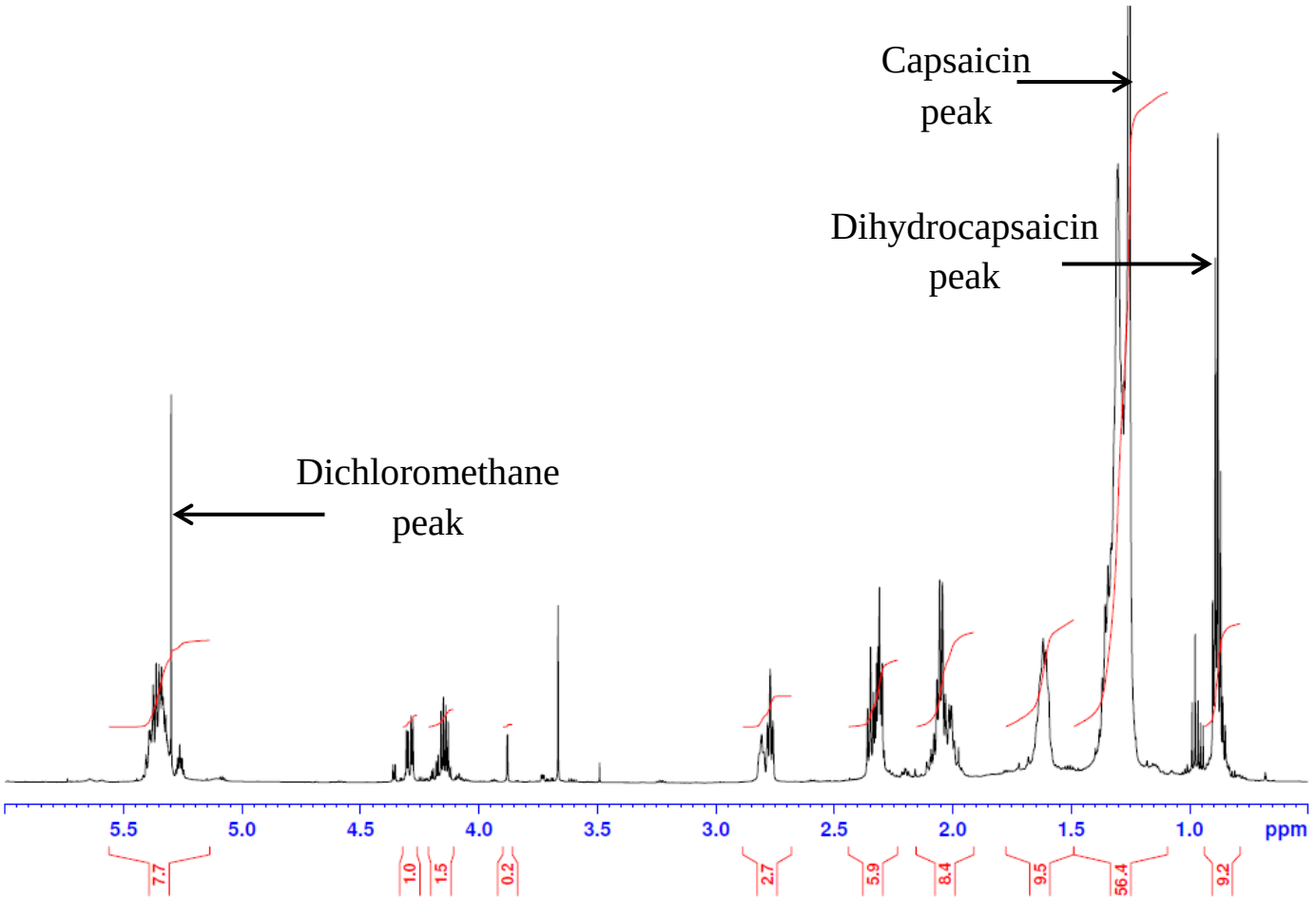


Figure 6b: Percentage estimation of capsaicin in purified extract by 1H-NMR. The doublet of the isopropyl group of capsaicin and dihydrocapsaicin, which occurred at 1.20 ppm and 0.80 ppm were used to approximate the relative quantities of the obtained extract. The recognisable peak areas for capsaicin and dihydrocapsaicin were matched against their respective standard curve to estimate their approximate percentages within the extract.

3.2 Physiological variables

The body weight and energy consumption of H rats were higher than the C rats both after 8 and 16 weeks. Body weight and energy intake were reduced by capsaicin in both CC and HC rats compared to C and H rats, respectively (Table 6). H rats consumed less food than C rats, and capsaicin treatment reduced food intake by approximately 30% in both CC and HC groups, respectively. There was no change in water consumption in the experimental and control groups. Hence, capsaicin had no effect on decreasing the energy intake found in the 25% fructose water solution. This indicates that capsaicin treatment reduces food consumption and, therefore, overall energy intake (Table 6 and Figure 7).

BMI and feed efficiency were higher in H rats than C rats. Capsaicin treatment improved feed efficiency in both HC and CC groups compared to H and C rats respectively. Capsaicin intervention lowered BMI in both HC and CC rats by 16% and 8% when compared to H and C rats, respectively. H rats gained more weight than their C counterparts during the 16 week protocol. Capsaicin treatment lowered the weight gained during the intervention period between weeks 8 and 16 by 92% in CC rats and 33% in HC rats. This indicates that capsaicin improves feed efficiency and reduces BMI and the weight gained, independent of the diet consumed.

Capsaicin intake was dependent on the quantity of food ingested, as it was placed in the food, thus permitting oral administration. Since CC rats consumed more food than HC rats, capsaicin intake was greater in CC rats (13.29±0.33 mg/kg body weight daily) than in HC rats (7.29±0.31 mg/kg body weight daily).

3.3 Body composition analysis

Abdominal circumference and fat pad weights were higher in H rats compared to C rats. Capsaicin treatment normalised abdominal fat deposition and prevented the increase in abdominal circumference in HC rats, as well decreasing abdominal fat deposition and circumference in CC rats

(Table 4). All components of abdominal fat (retroperitoneal, omental and epididymal fat pads) increased in H rats compared to their C counterparts, whilst capsaicin treatment reduced fat deposition in these areas in both the HC and CC groups when compared to H and C groups respectively. Hence, capsaicin treatment reduced abdominal fat deposition and, therefore, abdominal circumference measurements. Additionally, dorsal brown fat pads were compared in the different groups. Brown fat was higher in H rats compared to C rats. Capsaicin treatment did not change brown fat in either of the groups.

A dual-energy X-ray absorptiometer (DXA) was used to measure bone mineral content, bone mineral density, lean mass and fat mass of the rats. Total body fat mass was higher in H rats when compared to C rats. Capsaicin treatment lowered total body fat mass in both the HC and CC groups respectively. Lean mass was higher in H rats compared to C rats but capsaicin intervention did not change body lean mass. Additionally, bone mineral content and density were increased in the H rats compared to C rats. Capsaicin treatment did not change bone mineral density or content.

Kidney and spleen wet weights are given in Table 4. Wet weight increased for these organs in H rats compared to C rats. Capsaicin intervention did not alter the wet weights of these organs.

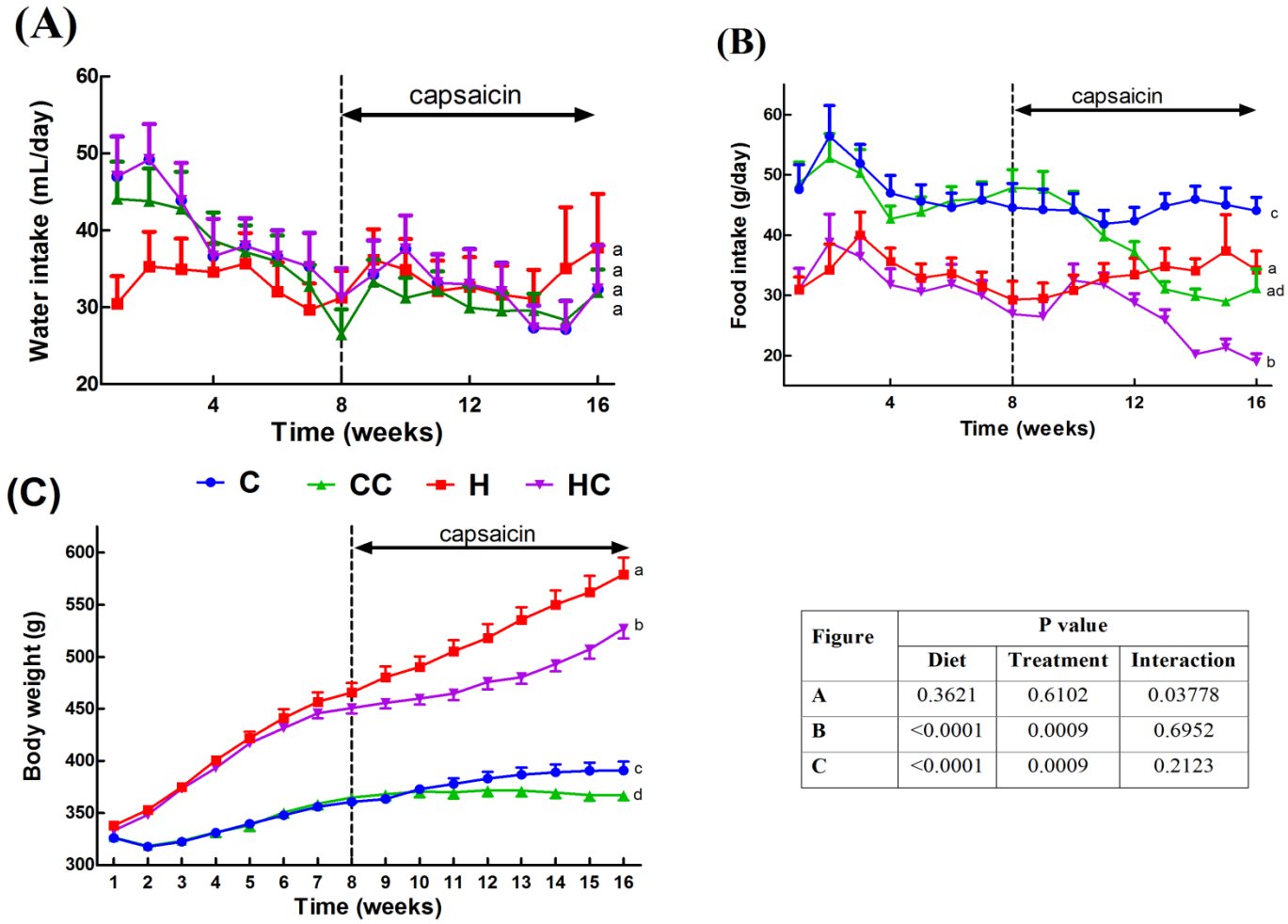


Figure	P value		
	Diet	Treatment	Interaction
A	0.3621	0.6102	0.03778
B	<0.0001	0.0009	0.6952
C	<0.0001	0.0009	0.2123

Figure 7: Effects of capsaicin on water intake (A), food intake (B) and body weight (B) in C, CC, H and HC rats over 16 weeks. Values are mean ± SEM, *n*=8-12. Means without a common letter differ, P<0.05. C, cornstarch diet-fed rats; CC, cornstarch diet-fed rats supplemented with capsaicin (13.3 mg/kg body weight/day); H, high-carbohydrate, high-fat diet-fed rats; HC, high-carbohydrate, high-fat diet-fed rats supplemented with capsaicin (7.3 mg/kg body weight/day).

Table 6: Effects of capsaicin on physiological variables and body composition

Variable	C	CC	H	HC	P value		
					Diet	Treatment	Interaction
Physiological variables							
Feed efficiency, g/kJ	0.11±0.01 ^{c}	0.05±0.01 ^{d}	0.33±0.02 ^{a}	0.29±0.02 ^{b}	<0.0001	0.0074	0.3526
Energy intake, kJ/day	518±14 ^{c}	408±10 ^{d}	725±17 ^{a}	584±14 ^{b}	<0.0001	<0.0001	0.2524
Capsaicin intake, mg/kg body weight/day	N/A	13.29±0.33 ^{b}	N/A	7.29±0.31 ^{a}	<0.0001	<0.0001	<0.0001
Bone mineral content (8 weeks), g	11.1±0.3 ^{b}	10.6±0.2 ^{b}	14.8±0.6 ^{a}	13.4±0.6 ^{a}	<0.0001	0.266	0.2893
Bone mineral density (8 weeks), g/cm^2	0.175±0.002 ^{b}	0.170±0.002 ^{ab}	0.177±0.003 ^{a}	0.183±0.003 ^{a}	0.0065	0.9427	0.0325
Total fat mass (8 weeks), g	49.1±6.7 ^{b}	53.9±5.0 ^{b}	170.5±17.9 ^{a}	126.5±11.6 ^{a}	<0.0001	0.0688	0.0255
Total lean mass (8 weeks), g	302.4±5.5 ^{a}	305.0±3.4 ^{a}	308.7±7.5 ^{a}	303.7±10.2 ^{a}	0.359	0.07412	0.8117
BMI, g/cm^2	0.62±0.02 ^{c}	0.57±0.01 ^{c}	0.86±0.02 ^{a}	0.73±0.01 ^{b}	<0.0001	<0.0001	0.091
Body weight gain, g	52±9 ^{c}	27±5 ^{d}	276±36 ^{a}	186±9 ^{b}	<0.0001	0.0056	0.1230
Abdominal circumference (8 weeks), cm	16.6±0.5 ^{bc}	15.5±0.1 ^{bc}	19.8±0.6 ^{a}	17.3±0.2 ^{ab}	<0.0001	0.4734	0.1578
Abdominal circumference (16 weeks), cm	17.7±0.4 ^{c}	14.3±0.1 ^{d}	24.3±0.5 ^{a}	19.0±0.2 ^{b}	<0.0001	<0.0001	<0.0001
Visceral adiposity (16 weeks), %	5.63±0.45 ^{c}	3.21±0.16 ^{d}	11.33±0.48 ^{a}	8.42±0.46 ^{b}	<0.0001	<0.0001	0.548
Dorsal brown fat (16 weeks), g	1.07±0.13 ^{b}	1.36±0.11 ^{ab}	1.57±0.07 ^{a}	1.55±0.1 ^{a}	0.2077	0.0023	0.1493
Tissue wet weight (16 weeks), mg/mm tibial length							
Retroperitoneal	219±22 ^{c}	69±11 ^{d}	706±67 ^{a}	424±31 ^{b}	<0.0001	<0.0001	0.192
Epididymal	116±10 ^{c}	97±8 ^{d}	324±20 ^{a}	219±16 ^{b}	<0.0001	0.0008	0.0793
Omental	116±17 ^{c}	71±6 ^{d}	315±19 ^{a}	204±15 ^{b}	<0.0001	<0.0001	0.0826
Total abdominal fat	451±43 ^{c}	238±16 ^{d}	1345±97 ^{a}	847±55 ^{b}	<0.0001	<0.0001	0.0669
Kidney	50.2±1.9 ^{b}	46.1±1.6 ^{b}	61.2±0.9 ^{a}	55.0±1.3 ^{a}	<0.0001	0.0575	0.7954
Spleen	15.0±1.0 ^{b}	14.2±0.8 ^{b}	20.1±0.9 ^{a}	19.1±0.8 ^{a}	<0.0001	0.7579	0.6079

Values are mean ± SEM, n=7-12. Means without a common letter differ, P<0.05. C, cornstarch diet-fed rats; CC, cornstarch diet-fed rats supplemented with capsaicin (13.3 mg/kg body weight/day); H, high-carbohydrate, high-fat diet-fed rats; HC, high-carbohydrate, high-fat diet-fed rats supplemented with capsaicin (7.3 mg/kg body weight/day).

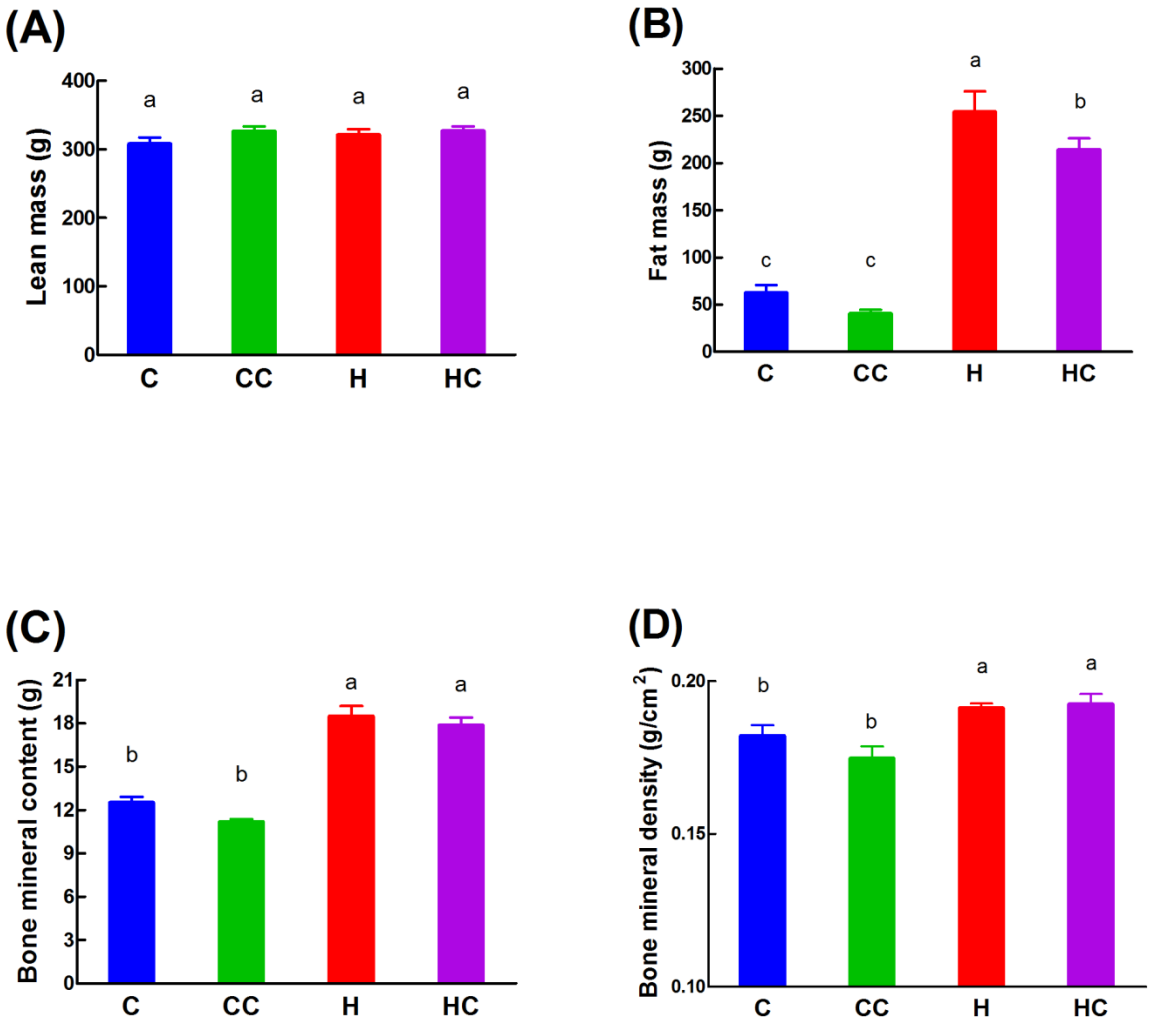


Figure	P value		
	Diet	Treatment	Interaction
A	0.4184	0.1579	0.4281
B	0.0391	<0.0001	0.5243
C	<0.0001	0.079	0.5006
D	0.0003	0.3574	0.1848

Figure 8: Effects of capsaicin on total body lean mass (A), fat mass (B), bone mineral content (C) and bone mineral density (D) in C, CC, H and HC rats at 16 weeks. Values are mean ± SEM, *n* = 7-8. Means without a common letter differ, P<0.05. C, cornstarch diet-fed rats; CC, cornstarch diet-fed rats diets supplemented with capsaicin (13.3 mg/kg body weight/day); H, high-carbohydrate, high-fat diet diet-fed rats; HC, high-carbohydrate, high-fat diet diet-fed rats supplemented with capsaicin (7.3 mg/kg body weight/day).

3.4 Metabolic variables

The effects of capsaicin on overnight fasting blood glucose concentrations and glucose sensitivity were tested through OGTT and presented as the area under the curve (AUC) (Figure 9A). Fasting basal blood glucose concentrations were higher in H rats than C rats. Capsaicin did not alter basal blood glucose concentration. The AUC for H rats was higher compared to C, CC and HC rats, thus indicating an impaired blood glucose tolerance in H rats. Capsaicin treatment reduced the AUC for both CC and HC rats when compared to H and C diets. Additionally, the AUC for HC rats was unchanged when compared to C rats (Figure 9A). Plasma concentrations of triglycerides and NEFA were elevated in H rats compared with C rats. There was no difference in plasma concentrations between H and C rats. Capsaicin treatment lowered the plasma concentrations of total cholesterol and triglycerides in HC and CC rats compared to H and C rats respectively (Table 7). However, capsaicin had no effect on plasma concentrations of NEFA. Hence, capsaicin reversed and normalised impaired glucose tolerance in HC rats and improved glucose utilisation in CC rats, as well as preventing increases in plasma concentrations of triglycerides and total cholesterol.

3.5 Liver structure and damage

H rats showed higher wet weight of the liver than C rats. Capsaicin treatment reduced the wet weight of the liver in both HC and CC groups respectively (Table 7). The effects of capsaicin on steatosis and inflammation in the liver are displayed in Figure 10. H rats showed considerably more steatosis and inflammatory cell migration than the C groups. Microscopically, there appeared to be minimal variation between the C and CC rats. However, capsaicin attenuated steatosis and decreased inflammatory cell migration in HC rats compared to H rats. Hence, capsaicin reduced the liver wet weight by improving hepatic structure.

Plasma activities of AST and ALT were higher in H rats than in C rats. Capsaicin treatment decreased both AST and ALT plasma activities in HC rats (Table 7). Capsaicin treatment did not

change AST and ALT plasma activities in CC rats when compared to C rats. Hence, capsaicin treatment normalised plasma activities of liver damage markers.

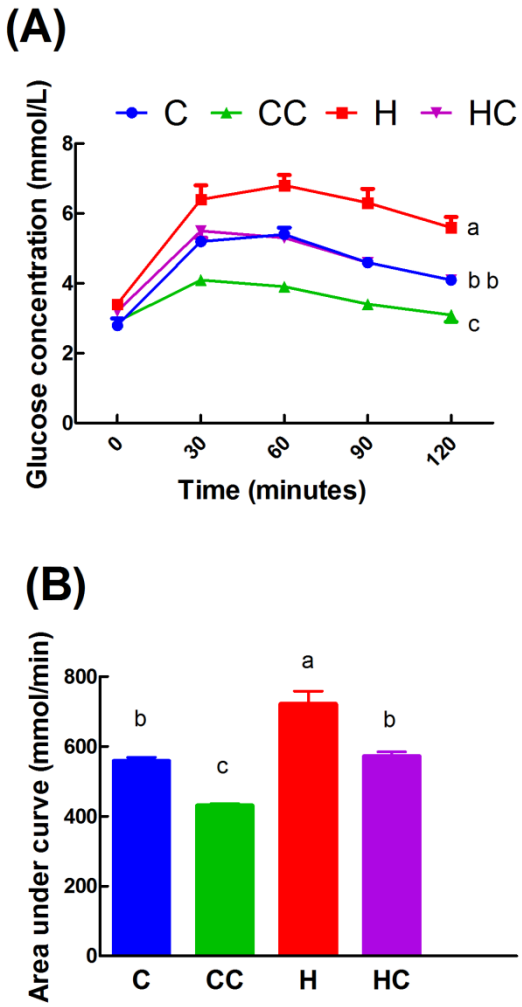


Figure	P value		
	Diet	Treatment	Interaction
A	<0.0001	<0.0001	0.5782
B	<0.0001	<0.0001	0.2151

Figure 9: Effects of capsaicin on plasma glucose concentrations (A) and area under the curve (B) in C, CC, H and HC rats at 16 weeks. Values are mean ± SEM, *n* = 7-12. Means without a common letter differ, P<0.05. C, cornstarch diet-fed rats; CC, cornstarch diet-fed rats supplemented with capsaicin (13.3 mg/kg body weight/day); H, high-carbohydrate, high-fat diet-fed rats; HC, high-carbohydrate, high-fat diet-fed rats supplemented with capsaicin (7.3 mg/kg body weight/day).

Table 7: Effects of capsaicin on plasma biochemistry and hepatic variables

					P value		
Variable	**C**	**CC**	**H**	**HC**	**Diet**	**Treatment**	**Interaction**
Plasma biochemistry							
Basal blood glucose concentration, mmol/L	2.8±0.2^{b}	2.9±0.1^{b}	3.4±0.1^{a}	3.2±0.1^{ab}	0.0007	0.6843	0.2266
Total cholesterol, mmol/L	2.1±0.1^{a}	1.5±0.1^{b}	1.9±0.1^{a}	1.5±0.1^{b}	0.3354	<0.0001	0.3354
Triglyceride, mmol/L	0.6±0.1^{c}	0.5±0.1^{c}	2.1±0.2^{a}	1.4±0.2^{b}	<0.0001	0.0131	0.0572
Non-esterified fatty acids, mmol/L	1.7±0.2^{b}	1.5±0.1^{b}	3.9±0.3^{a}	3.8±0.5^{a}	<0.0001	0.6361	0.8745
Hepatic variables							
Liver wet weight, mg/mm tibia	230±14^{c}	202±9^{c}	399±22^{a}	294±12^{b}	<0.0001	0.0005	0.0561
Plasma ALT, U/L	40±5^{b}	35±2^{b}	56±6^{a}	38±2^{b}	0.0255	0.0088	0.1198
Plasma AST, U/L	109±11^{b}	102±10^{b}	192±27^{a}	122±16^{b}	0.0322	0.005	0.074

Values are mean ± SEM, *n*=8-12. Means without a common letter differ, P<0.05. C, cornstarch diet-fed rats; CC, cornstarch diet-fed rats supplemented with capsaicin (13.3 mg/kg body weight/day); H, high-carbohydrate, high-fat diet-fed rats; HC, high-carbohydrate, high-fat diet-fed rats supplemented with capsaicin (7.3 mg/kg body weight/day).

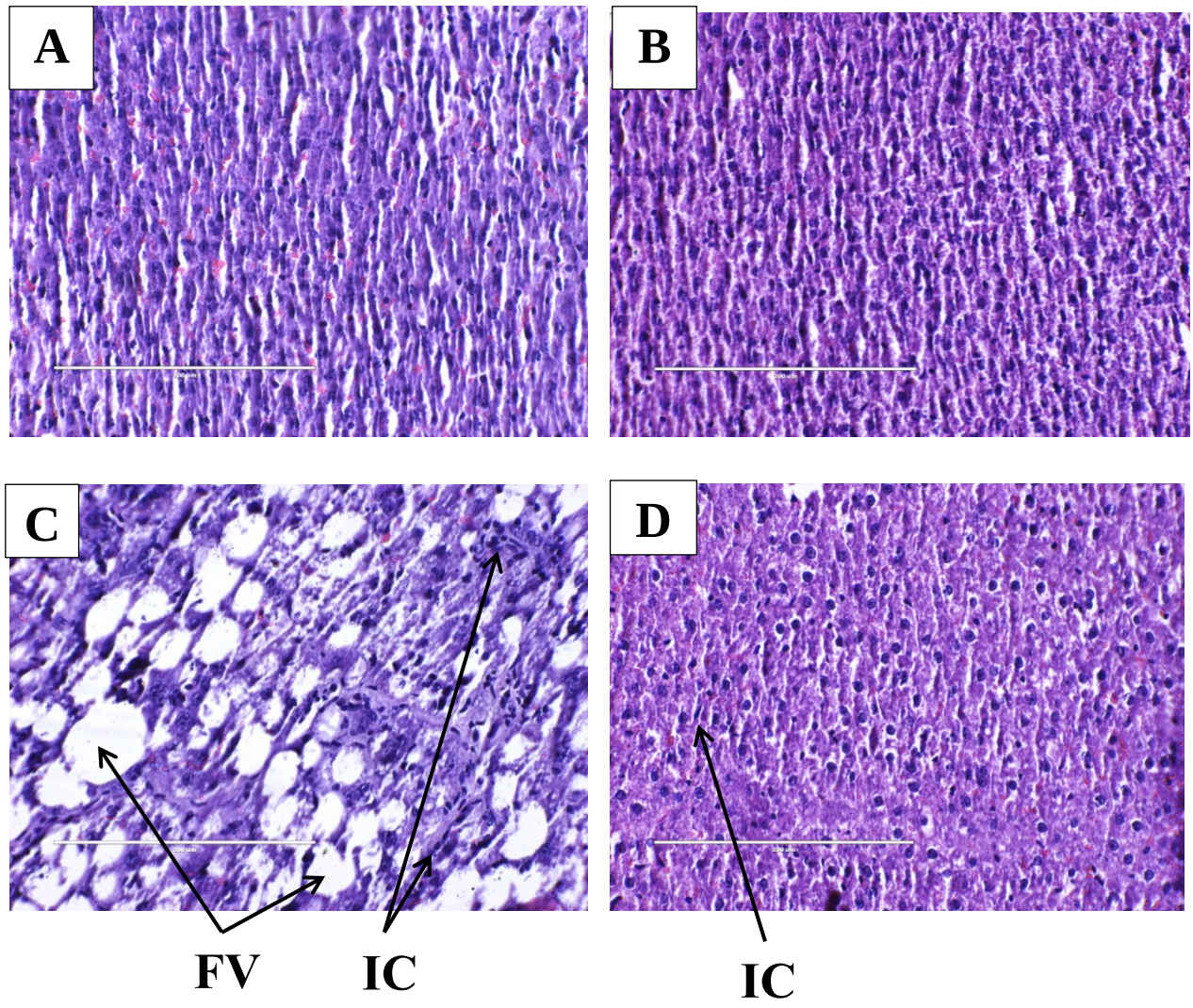


Figure 10: Effects of capsaicin on fat deposition and inflammation in the liver. A-D represents haematoxylin and eosin staining of the liver showing fat deposition (marked as 'FV') and inflammatory cells (marked as 'IC') (20x). C, cornstarch diet-fed rats (A); CC, cornstarch diet-fed rats supplemented with capsaicin (7.3 mg/kg body weight/day) (B); H, high-carbohydrate, high-fat diet-fed rats (C); HC, high-carbohydrate, high-fat diet-fed rats supplemented with capsaicin (13.3 mg/kg body weight/day) (D).

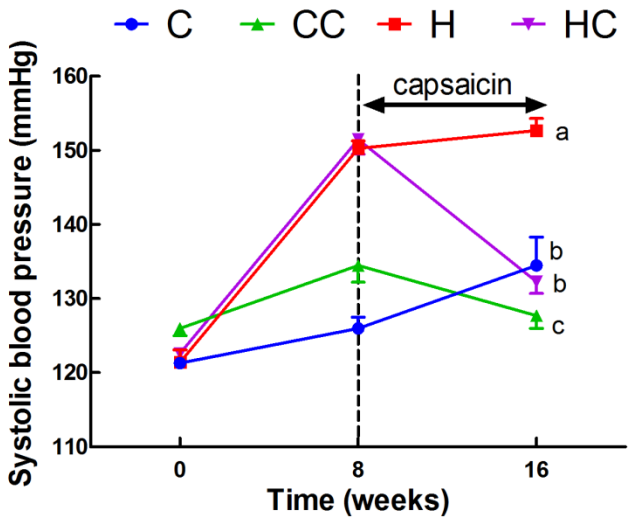
3.6 Cardiac structure and function

Systolic blood pressure was measured at 0, 8 and 16 weeks and is presented in figure 11. By week 8, H feeding increased systolic blood pressure to 150±1 mmHg in H rats and 151±2 mmHg in HC rats compared to 126±2 mmHg in C rats and 134±2 mmHg. At this point within the protocol, there were no differences in systolic blood pressure between H and HC rats. However, an 8 mmHg difference in systolic blood pressure was evident between CC rats and C rats, where the systolic blood pressure in CC rats was higher than C rats. Upon completion of the 16 week protocol, the systolic blood pressure of the H rats and C rats had increased from the 8 week values. However, the systolic blood pressure of both the HC and CC rats had declined considerably since the eighth week. Capsaicin exerted a greater effect on systolic blood pressure in rats that are H fed compared to C fed rats. Therefore, capsaicin treatment attenuated hypertension in H fed rats and improved systolic blood pressure in C fed rats.

The vascular reactivity of the isolated thoracic aorta to acetylcholine, noradrenaline and sodium nitroprusside was measured and is shown in figure 13. There was no difference amongst all groups when the aorta was subjected to noradrenaline and sodium nitroprusside. There was no difference between H and C rats when the aorta was exposed to noradrenaline. However, HC and CC rats demonstrated an increased acetylcholine-induced relaxation. This increased relaxation was particularly apparent in the HC group when compared to the H group. Hence, capsaicin treatment reversed the endothelial damage caused by the H diet, thus returning vascular relaxation to normal.

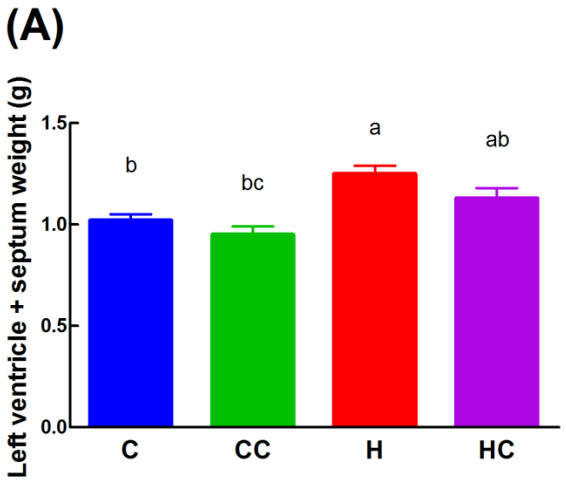
The isolated Langendorff heart preparation was used to assess the stiffness of the heart, defined by the left ventricular diastolic stiffness constant, κ (Figure 12C). The diastolic stiffness was increased in H rats compared to C rats. Capsaicin treatment lowered diastolic stiffness in HC rats when compared to H rats, to a value similar to C rats. There was no difference in diastolic stiffness between CC and C rats. The effects of capsaicin on inflammatory cell migration and collagen deposition within the heart are displayed in Figures 14 and 15. H rats demonstrated inflammatory

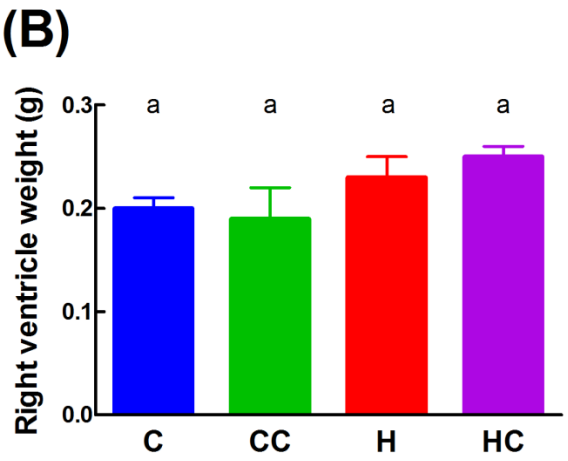
cell migration and collagen deposition within the heart compared to C groups. Microscopically, there appeared to be minimal variation between the C and CC rats. However, capsaicin attenuated inflammatory cell migration and reduced collagen deposition in HC rats compared to H rats. Additionally, H rats increased left ventricular and septum mass, as well as an increased right ventricular mass compared to C rats (Figures 12A and B). There were no differences between the mass of the right ventricle in CC and HC rats. However, capsaicin treatment decreased the mass of the left ventricle and septum in both CC and HC rats compared to H and C groups. Hence, capsaicin treatment normalised systolic blood pressure and hence stiffness of the heart, inflammatory cell migration and collagen deposition, which in turn, decreased the mass of the heart in the presence of H feeding.



Time (weeks)	P value		
	Diet	Treatment	Interaction
0	0.1167	0.0804	0.0974
8	<0.0001	0.0603	0.1396
16	<0.0001	<0.0001	0.0045

Figure 11: Effects of capsaicin on systolic blood pressure in C, CC, H and HC rats over 16 weeks. Values are mean ± SEM, *n* =5-10. Means without a common letter differ, P<0.05. C, cornstarch diet-fed rats; CC, cornstarch diet-fed rats supplemented with capsaicin (13.3 mg/kg body weight/day); H, high-carbohydrate, high-fat diet-fed rats; HC, high-carbohydrate, high-fat diet-fed rats supplemented with capsaicin (7.3 mg/kg body weight/day).





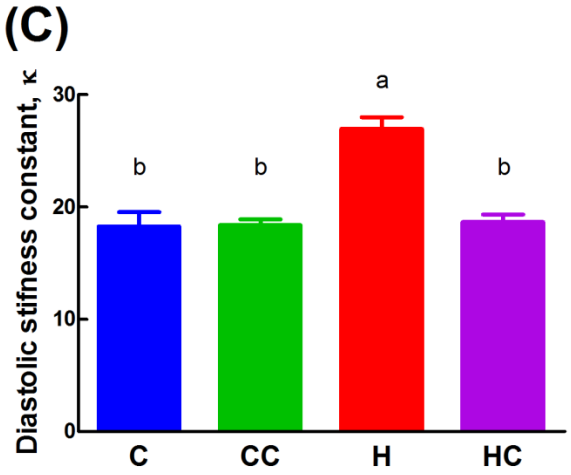


Figure	P value		
	Diet	Treatment	Interaction
A	<0.0001	0.0305	0.5560
B	0.0334	0.8067	0.4646
C	<0.0001	<0.0001	<0.0001

Figure 12: Effects of capsaicin on systolic left ventricle and septum weight (A), right ventricle weight (B) and diastolic stiffness (C) in C, CC, H and HC rats at 16 weeks. Values are mean ± SEM, *n* =5-10. Means without a common letter differ, P<0.05. C**,** cornstarch diet-fed rats; CC, cornstarch diet-fed rats diets supplemented with capsaicin (13.3 mg/kg body weight/day); H**,** high-carbohydrate, high-fat diet diet-fed rats; HC, high-carbohydrate, high-fat diet diet-fed rats supplemented with capsaicin (7.3 mg/kg body weight/day).

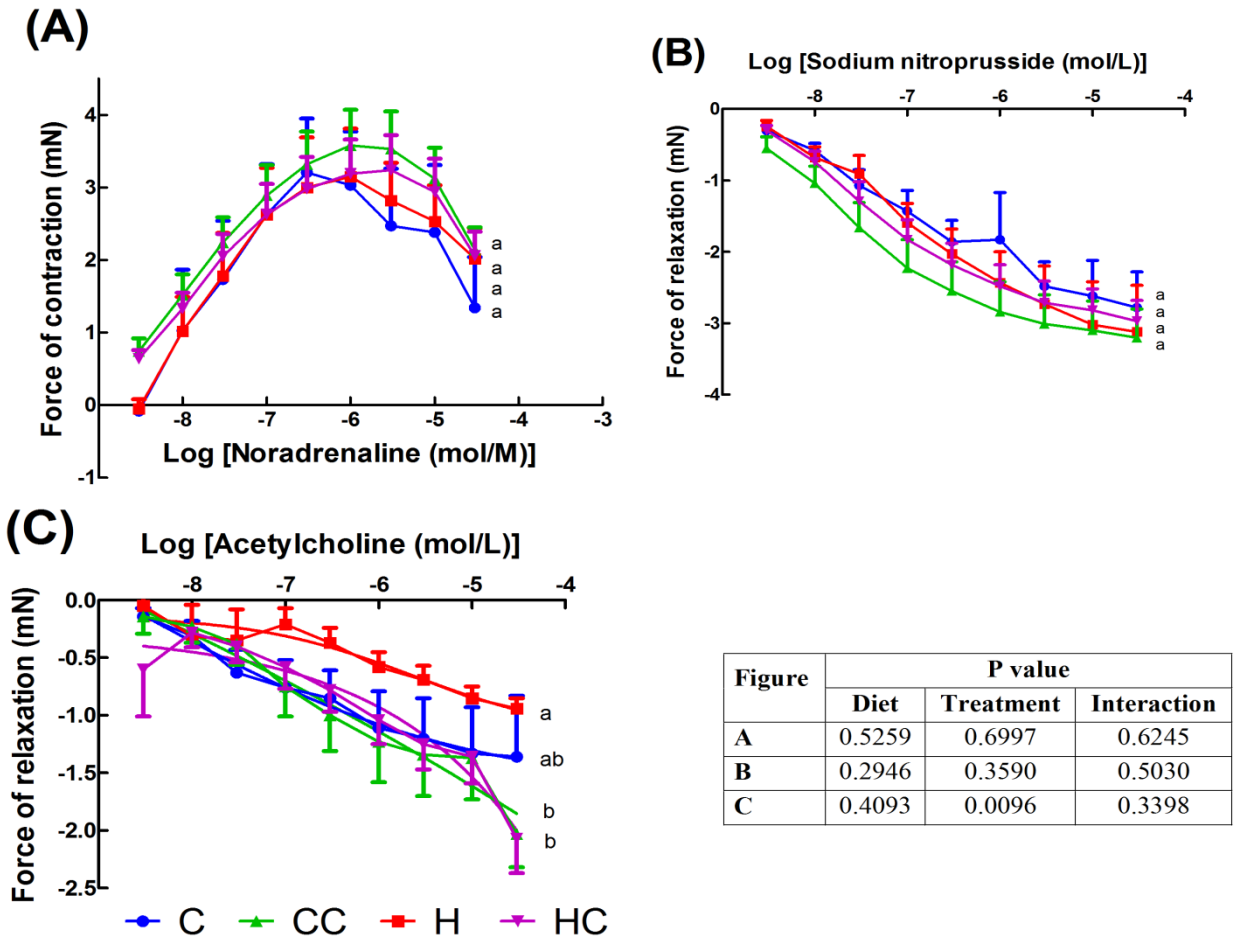


Figure	P value		
	Diet	Treatment	Interaction
A	0.5259	0.6997	0.6245
B	0.2946	0.3590	0.5030
C	0.4093	0.0096	0.3398

Figure 13: The effect of capsaicin on noradrenaline-induced contraction (A), sodium nitroprusside-induced relaxation (B) and acetylcholine-induced relaxation (C) in thoracic aortic rings from C, CC, H and HC rats at 16 weeks. Values are mean ± SEM, *n* =5-10. Means without a common letter differ, P<0.05. C**,** cornstarch diet-fed rats; CC, cornstarch diet-fed rats diets supplemented with capsaicin (13.3 mg/kg body weight/day); H**,** high-carbohydrate, high-fat diet diet-fed rats; HC, high-carbohydrate, high-fat diet diet-fed rats supplemented with capsaicin (7.3 mg/kg body weight/day).

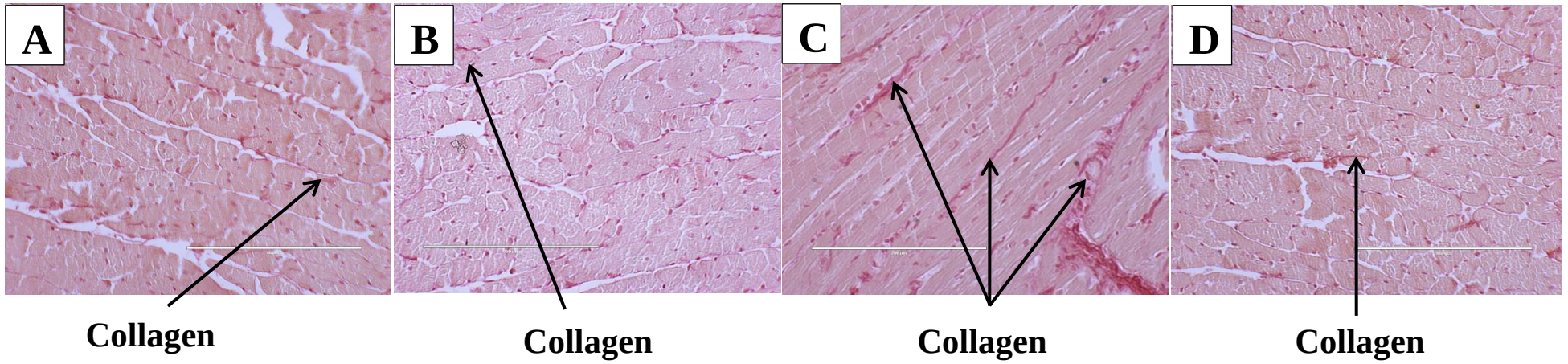


Figure 14: Effects of capsaicin on collagen deposition in the heart. A-D represents picrosirius red staining of the heart showing collagen deposition (20x). C, cornstarch diet-fed rats (A); CC, cornstarch diet-fed rats supplemented with capsaicin (13.3 mg/kg body weight/day) (B); H, high-carbohydrate, high-fat diet-fed rats (C); HC, high-carbohydrate, high-fat diet-fed rats supplemented with capsaicin (7.3 mg/kg body weight/day) (D).

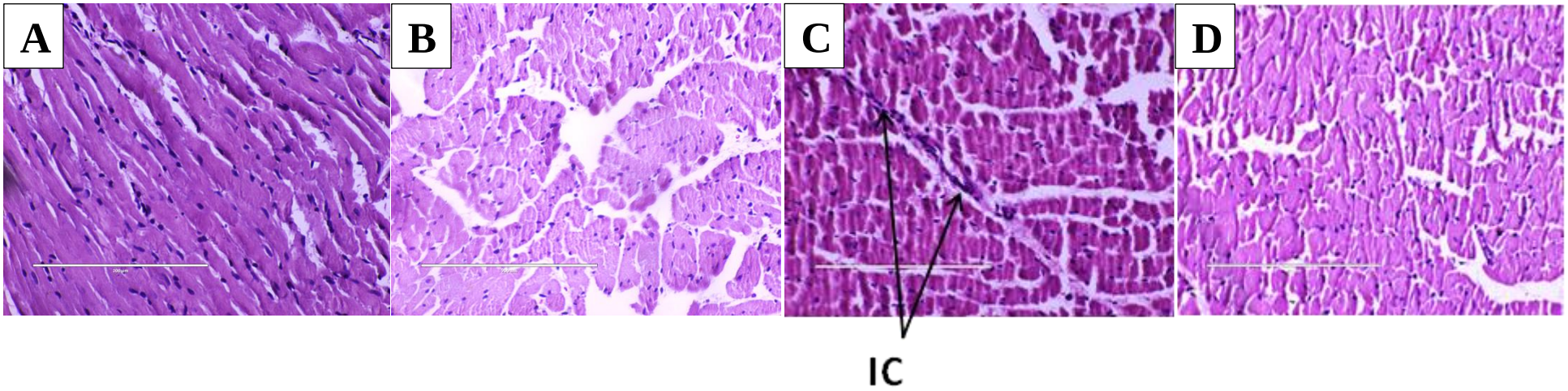


Figure 15: Effects of capsaicin on inflammation in the heart. A-D represents haematoxylin and eosin staining of the heart showing inflammatory cells (marked as 'IC') (20x). C, cornstarch diet-fed rats (A); CC, cornstarch diet-fed rats supplemented with capsaicin (13.3 mg/kg body weight/day) (B); H, high-carbohydrate, high-fat diet-fed rats (C); HC, high-carbohydrate, high-fat diet-fed rats supplemented with capsaicin (7.3 mg/kg body weight/day) (D).

3.7 Gastrointestinal smooth muscle function

In the ileum, there was no difference in acetylcholine-induced contraction between H and C rats (Figure 16A). However, CC and HC rats demonstrated increased contractile responses to acetylcholine. In the colon organ bath, there were no differences between all of the groups (Figure 16B). Hence, capsaicin improved contractility only in the ileum.

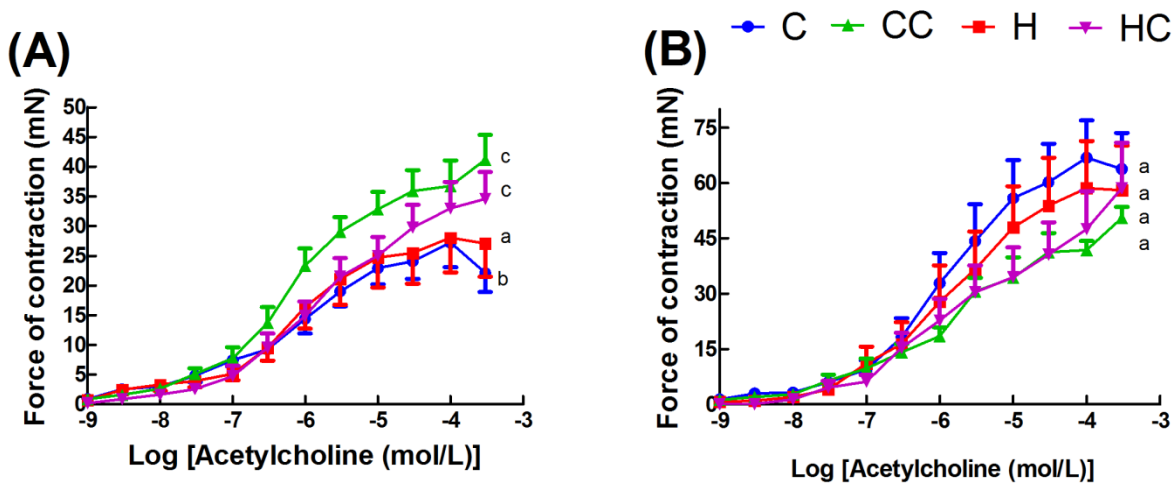


Figure	P value		
	Diet	Treatment	Interaction
A	<0.0001	<0.0001	0.5782
B	<0.0001	<0.0001	0.2151

Figure 16: The effect of capsaicin on acetylcholine-induced contraction in ileum (A) and colon (B) rings from C, CC, H and HC rats at 16 weeks. Values are mean ± SEM, *n* =5-10. Means without a common letter differ, P<0.05. C**,** cornstarch diet-fed rats; CC, cornstarch diet-fed rats diets supplemented with capsaicin (13.3 mg/kg body weight/day); H**,** high-carbohydrate, high-fat diet diet-fed rats; HC, high-carbohydrate, high-fat diet diet-fed rats supplemented with capsaicin (7.3 mg/kg body weight/day).

Chapter 4: Discussion

4.1 Capsaicin extraction and purification

In this study, capsaicin was first isolated from chillies and then characterised before its administration to rats fed either a C or H diet. The streamlined processes for the extraction and purification of capsaicin from various chilli cultivars generated a yield of 1.42%. A yield of approximately 0.5 – 2.0% from different chillies has been reported using similar procedures as in this study (Huang et al. 2000; Peña-Alvarez et al. 2009; Koleva Gudeva et al. 2013; Goci et al. 2014; Ashwini et al. 2015a).

The presence of capsaicin and an estimate of its proportion were determined by ^{1}H-NMR spectroscopy. Protons grouped on a specific carbon or other atoms possess different resonances between α and β-spin states than protons on other carbon atoms, which is dependent on their given environments (Bertini et al. 2012; Jacobsen 2016). The distinctive structural activities convey different electron densities around the proton nuclei or different local magnetic environments, thus resulting in a unique resonance (Wagner et al. 2011; Bertini et al. 2012; Jacobsen 2016). Additionally, the resonance energy of a group of protons on a specific carbon atom will be coupled to the spin states of the protons situated on neighbouring carbons (Bertini et al. 2012; Jacobsen 2016). This results in what is termed a 'classical' splitting rule (n+1), where a single or group of protons on a specific carbon are split into a total of (n+1) peaks, where n is the number of protons on carbons adjacent to the specific carbon (Bertini et al. 2012; Jacobsen 2016). Therefore, if n=0, the group of protons appears as a singlet; if n=1, the protons appear as a doublet; if n=2, the protons appear as triplet and so on. This method was used to provide structural evidence for the compounds in the extract. Hence, the presence of capsaicin was confirmed, as was the presence of dihydrocapsaicin and remnant dichloromethane as the solvent. The spectra obtained shows similar values to the literature that have used ^{1}H-NMR spectroscopy to determine the structure of compounds isolated from chillies (Lin et al. 1993; Yao et al. 1994; Wagner et al. 2011).

This study described approximate percentages of 86% capsaicin and 14% dihydrocapsaicin in the final extract. This proportion resembles that of commercially sold natural capsaicin, which typically reports a ratio of 65-80% capsaicin to 20-35% dihydrocapsaicin (Lin et al. 1993; Yao et al. 1994; Betts 1999; Abdel-Salam 2014). These values were obtained from the doublet of the isopropyl group of capsaicin and dihydrocapsaicin, which occurred between approximately 1.10 ppm and 0.80 ppm. These recognisable peak areas for capsaicin and dihydrocapsaicin were matched against their respective standard curves to estimate their approximate percentages within the extract (Yao et al. 1994). Higher purification values of capsaicin have been reported in the literature, ranging from 85%-98%, where extended purification assays using high-performance liquid chromatography and gas chromatography–mass spectrometry have been implemented (Yao et al. 1994; Betts 1999; Peña-Alvarez et al. 2009). Finally, the presence of dichloromethane was expected as it is commonly used in other purification procedures as a solvent and its presence is typically detected (Gottlieb et al. 1997). However, it is unknown what percentage of a solvent would be expected to be present following the purification process, as previous studies have not reported the quantity of solvents or artefacts present from their purification or clean-up process (Gottlieb et al. 1997; Niedzialkowska et al. 2016). Hence, it is not clear as to what is a common proportion of solvent observed during capsaicin extraction and purification, as well as the effect that the presence of a low percentage of solvent within an extract may convey on a biological study.

4.2 Capsaicin dosage

Acute high-doses of capsaicin administered orally, intraperitoneally, subcutaneously, intrathecally and intravenously convey detrimental effects to an animal depending on the dose, where the greater doses produce more serious adverse effects (Glinsukon et al. 1980; Gamse 1982; Surh & Sup Lee 1995; Rüttimann et al. 2009; Zhang & Ritter 2012). Adverse effects include neurotoxicity, hormonal imbalances, inflammation, severe cardiorespiratory depression or excitation, temporary paralysis, cancer and death at doses of ranging from 50mg/kg to 10g/kg body weight (Glinsukon et

al. 1980; Gamse 1982; Surh & Sup Lee 1995; Rüttimann et al. 2009; Zhang & Ritter 2012). However, low-dose capsaicin administered by different administration routes, in particular the oral route, have demonstrated positive health outcomes and have been extensively reviewed elsewhere (Abdel-Salam 2014). These studies typically report a dietary dose of capsaicin equivalent of 0.005% to 0.025% of an animal's dietary food intake as conveying positive health benefits (Mózsik et al. 2005; Zvara et al. 2005; Leung 2008; Kang et al. 2010; Yang et al. 2010; Xu et al. 2011; Wang et al. 2014; Chen et al. 2015; Kang et al. 2016). Hence, an initial dose of 5mg/kg body weight/day of dietary capsaicin was proposed for this study. This equated to approximately 0.015% of the total daily dietary intake of rats. Therefore 0.015% of capsaicin was placed into the food, as outlined in section 2.3 Rats, experimental groups and housing.

The daily dosage is proportional to the amount of food consumed by the rats. Upon the initial period of treatment, the amount of food being ingested did not differ from the control groups. However, as the protocol progressed, the amount of food ingested by the CC and HC rats decreased, which, in turn, meant that the dose of capsaicin decreased. Additionally, as the protocol progressed and the weight of the rats increased, the dose of capsaicin was reduced. The CC rats ingested approximately 13.3 mg/kg of body weight/day and the HC rats approximately 7.3 mg/kg of body weight/day, more than that initially expected. The dose ingested by HC and CC equates to approximately 97.2 mg/day or 1.62 mg/kg of body weight/day for a 60 kg human based on the body surface area rule described by Reagan-Shaw et al. (2008). This dose corresponds to the daily intake of capsaicin described in the rural Thai population, who report a 10.3-17.9% incidence of diet-induced obesity, which is significantly less than westernised countries, such as Australia (Aekplakorn et al. 2007; Leung 2008). Hence, future studies may utilise chilli as a whole food at a rate as consumed by the rural Thai population to study the effects that capsaicin may convey on diet-induced obesity and metabolic syndrome.

4.3 Central obesity and the development of metabolic syndrome

The risk factors of metabolic syndrome include central obesity, hypertension, decreased blood HDL, elevated fasting glucose and elevated blood triglycerides concentrations (Table 1) (Harris 2013). These features increase the likelihood of developing non-alcoholic fatty liver disease, cardiovascular disease and type 2 diabetes (Harris 2013). Central obesity is the predominant factor leading to metabolic syndrome diagnosis in Australia (Harris 2013). Nearly half of the Australian adult population and over 70% of people under 17 do not meet the minimum daily physical activity requirements (Tolhurst et al. 2016). Over 40% of Australians acquire their daily energy intake from an energy-dense westernised-diet high in both saturated fats and simple carbohydrates (McAllister et al. 2009; Tolhurst et al. 2016). Hence, it is clear that a large part of the obesity problem in Australia results from an increase in energy intake compared to energy output. The protocol of this study not only mimics the westernised-diet by administration of the H diet, but it also provides no structured exercise to animals throughout the course of the 16 weeks. This animal model, therefore, mirrors the lifestyle that is prevalent in Australia and the associated development of obesity and metabolic syndrome that results from these lifestyle choices.

Central obesity and increased body weight were evident by week 8 of the protocol. H fed rats had developed an increased fat mass and abdominal circumference in comparison to their C fed counterparts. The abdominal circumference, which is a primary marker used clinically to demonstrate central obesity, of the H fed rats continued to increase throughout the course of the 16 weeks (Harris 2013). Upon completion of the protocol, the H diet successfully induced the metabolic syndrome, not only evidenced by central obesity, but also by hypertension, impaired glucose tolerance and an elevated plasma concentration of triglycerides. Consequential changes of diet-induced obesity were also observed, including non-alcoholic fatty liver disease, demonstrated by deranged biochemical markers, steatohepatitis and an increased liver wet weight; and cardiovascular remodelling, indicated by increased myocardial wet weight, inflammatory cell

migration, collagen deposition, systolic blood pressure and left ventricular diastolic stiffness, as well as reduced endothelial function.

H rats also increased BMI, which is commonly used to categorise overweight and obesity. The increased abdominal circumference, weight gain and BMI were due to enlarged visceral adiposity evidenced by increased fat deposition in omental, retroperitoneal and epididymal fat pads, as well as an overall increase in fat mass by DXA. These markers were reduced in rats that were treated with low-dose dietary capsaicin. These findings are in keeping with the literature (Leung 2008; Kang et al. 2010; Chen et al. 2015). However, to my knowledge, this is the first study to demonstrate a decrease in BMI, weight, abdominal circumference, visceral adiposity, total fat mass and abdominal fat pad weight with low-dose dietary capsaicin in healthy subjects.

The increase in visceral or central adiposity, rather than the general increase in adiposity throughout the rest of the body, is responsible for the metabolic consequences of obesity (de Souza et al. 2012). However, the reasons why this occurs remain elusive, as its development is multi-faceted and complex. Central obesity is described as an increase in adiposity, low-grade inflammation, increased neurogenic tone and hormonal imbalances, as well as dysbiosis (Moran & Shanahan 2014; Bauer et al. 2016). Central obesity may result from postprandial blood flow distributing an increase in absorbed fat nutrients favourably to the adipose within the viscera (Leung 2008). Ingested fats are first sampled within the intestinal lumen prior to absorption and distribution to various adipose sites via the systemic circulation (Leung 2008). The intestinal lumen is lined with an epithelium rich in nutrient-sensing cells, such as enteroendocrine cells, which are responsible for initiating a majority of signalling and communication within the gut-brain axis in response to preabsorptive nutrients (Gribble & Reimann 2016). The apical plasmalemma of these cells are covered in microvilli, which open to and directly contact the luminal contents (Gribble & Reimann 2016). Additionally, vagal fibres extend into the lamina propria of the intestinal villa, terminate at the basolateral plasmalemma of enteroendocrine cells and express receptors for gut hormones such

as ghrelin, leptin, cholecystokinin (CCK), pancreatic peptide YY_{3-36} (PYY) and GLP1, thus leading to receptor activation and subsequent neuronal stimulation (Dockray 2013; Bauer et al. 2016). Additionally, when food enters the stomach in the form as a bolus, the stomach becomes stretched and triggers a feedback-loop to the brain by vagal afferents to cease eating (Cooke & Clark 1976; Suzuki et al. 2012). Chronic consumption of a hypercaloric diet, in particular a diet high in fat, is correlated to decreased vagal sensitivity to gut peptides, chemicals and distension (Daly et al. 2011). This results in impaired intestinal nutrient-sensing and energy homeostasis by an increased energy intake, thus contributing to the obesogenic state (Daly et al. 2011). It would be expected that these characteristics would have been prevalent in H-fed rats.

Vagal afferents express TRPV1, commonly referred to as the capsaicin receptor (Ono et al. 2011). Dietary capsaicin and capsaicin analogues, such as capsiate, bind to TRPV1 expressed on vagal afferents and cause an influx of cytosolic Ca^{2+}, thus causing neuronal excitation and activation of the gut-brain axis (Caterina et al. 1997; Ono et al. 2011; Hall 2015). Therefore, capsaicin may cause an increase in vagal firing, which results in an increased transmission of sensory information from the gut to the brain. The information conveyed by vagal afferents converges in the nucleus tractus solitarius of the dorsal vagal complex within the brainstem before being integrated and processed within the hypothalamus (Craig 1996; Schwartz et al. 2000). The hypothalamic arcuate nucleus responds to peripheral and central appetite signals via tightly-regulated neurotransmitter release from two separate neuronal populations, pro-opiomelanocortin and agouti-related protein neurons. Agouti-related protein neurons release the inhibitory neurotransmitters, agouti-related protein and neuropeptide Y (Cone et al. 2001; Suzuki et al. 2012; Buhmann et al. 2014). These neurotransmitters stimulate hunger and appetite, as well as decrease energy expenditure, thus contributing to excessive food consumption and weight gain (Dryden et al. 1995; Ollmann et al. 1997; Enriori et al. 2007). However, pro-opiomelanocortin neurons in the lateral arcuate nucleus release pro-opiomelanocortin, which stimulates the release of α-melanocortin-stimulating hormone and cocaine-and-amphetamine-regulated transcript (Suzuki et al. 2012). These neurotransmitters are

antagonistic of agouti-related protein and neuropeptide Y and decrease appetite and hunger, thus inhibiting food intake, as well as increasing energy expenditure, thus contributing to weight loss (Cowley et al. 2001; Nakhate et al. 2011; Bauer et al. 2016). Therefore, this provides a valid pathway as to how capsaicin may induce its effects within the diet-induced obese rat (Figure 17). This study did not evaluate this neurophysiological pathway so that this pathway can only be theorised to provide a valid means of how capsaicin may have exerted its effects from accepted literature, so further studies are required to demonstrate this. However, in support of this mechanism are studies that have demonstrated that rats with vagal ablation or vagotomy, in addition to TRPV1 deficiency, do not respond to capsaicin, do not increase intracellular Ca^{2+} concentrations, do not convey increased activity to the nucleus tractus solitarius or arcuate nucleus, display hyperphagia and an increase in weight (Rong et al. 2004; Bielefeldt & Davis 2008; Daly et al. 2011; Ono et al. 2011).

Increased vagal firing within rats treated with low-dose dietary capsaicin has been correlated to BAT activation (Kawabata et al. 2009; Ono et al. 2011). These authors suggest that TRPV1 activation in afferents supplying the gastrointestinal tract stimulate vagal nerve activity, which consequently triggers selective sympathetic neurons supplying BAT. Both studies demonstrated increased vagal activity and sympathetic neuron firing to BAT (Kawabata et al. 2009; Ono et al. 2011). Ludy et al. (2011) suggest that there is an interaction between sympathetic nervous system activity and food consumption whereby when this activity increases, consumption declines. Hence, enhanced sympathetic activity may increase satiety, thus reducing energy consumption, and increase BAT stimulation which, as demonstrated by Ono et al. (2011) and Kawabata et al. (2009), increases thermogenesis, metabolism and energy expenditure. My study found that there were differences in food consumption and, therefore, energy intake between rats treated with low-dose dietary capsaicin independent of the diet consumed. However, it did not identify changes in BAT wet weight in rats treated with capsaicin. It would have been expected to show changes in BAT wet weight if there were enhanced BAT activity. Whilst a change in BAT weight was not identified,

BAT activity cannot be excluded from improving visceral adiposity, a decreased food intake and a lower energy intake. This is so, as this study did not incorporate resting energy metabolism and indirect calorimetry measurements. Additionally, this study did not look directly at the molecular physiology associated with BAT activation. Markers such as thermogenin, which are located in the mitochondria of BAT and responsible for the uncoupling of oxidative phosphorylation thus resulting in thermogenesis and increased energy expenditure, could be used in addition to BAT wet weight measurements to highlight BAT functionality in future studies (Kozak & Anunciado-Koza 2008).

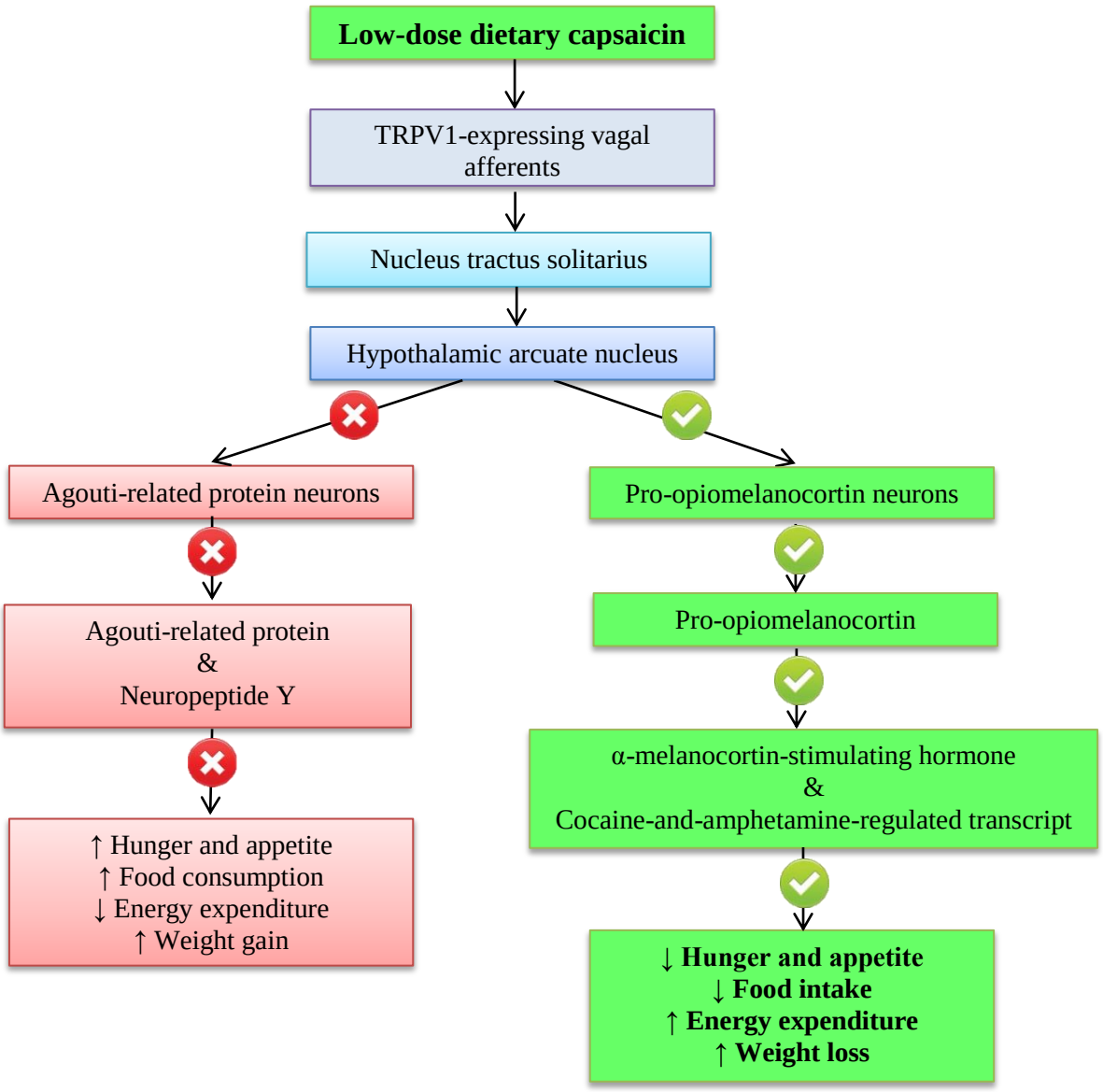


Figure 17: Proposed pathways of mechanisms by which low-dose dietary capsaicin reduces weight gain and promotes weight loss.

4.4 Glucose impairment and satiety

My study has demonstrated, as mentioned previously, improvements in feed efficiency and reductions in food consumption and therefore energy intake in both HC and CC rats. Hence, dietary capsaicin may exhibit its effects by increasing satiety and therefore preventing excess weight gain and adiposity. Additionally, dietary capsaicin attenuated the glucose intolerance in H rats and improved glucose tolerance in CC rats compared with C rats. These effects may result from enhanced vagal activity, as described above or via the release of GLP1. Dietary capsaicin ameliorated glucose dysregulation in conjunction with increased plasma and ileum concentrations of GLP1 in mice fed a high-fat diet (Wang et al. 2012). GLP1 is a gastrointestinal hormone released by enteroendocrine cells within the ileum, duodenum and colon 15 minutes postprandial (Elliott et al. 1993; Adam & Westerterp-Plantenga 2005). GLP1 binds to the GLP1 receptor, which is a GPCR located extensively throughout the central nervous system, gastrointestinal tract and pancreas (Larsen et al. 1997; Yamato et al. 1997). GLP1 subsequently decreased glucagon expression, upregulated insulin release and stimulated the satiety centres within the brain (Abbott et al. 2005; Wang et al. 2012). These effects were not observed when TRPV1 knockout mice ingest dietary capsaicin (Wang et al. 2012). Hence, dietary capsaicin treatment may increase satiety and improve glucose tolerance and utilisation via GLP1 release. However, the exact mechanism that capsaicin-induced activation of TRPV1 stimulates GLP1 release remains elusive. It is hypothesised that GLP1 release occurs in response to intracellular Ca^{2+} influx within vagal afferents, enteroendocrine cells and pancreatic β-cells, which is mediated by the activation of TRPV1 by low-dose dietary capsaicin (Wang et al. 2012). Further, a recent study demonstrated increased concentrations of GLP1 and gastric inhibitory peptide, as well as diminished ghrelin concentrations in healthy human subjects given low-dose dietary capsaicin (Kang et al. 2016). However, this short-term study did not note any changes in satiety, food intake or glucose concentrations. Kang et al. (2016) additionally reported a lower concentration of lipopolysaccharide-binding protein and faecal Gram-negative bacteria, which are both associated with increased neurogenic tone and chronic low-grade

inflammation, both of which are hallmarks of obesity (Moran & Shanahan 2014). This provides further complexity as to how dietary capsaicin may exert its effects on satiety and glucose tolerance. Whilst the mechanisms involved are important to study for future endeavours, my study has demonstrated that low-dose dietary capsaicin treatment diminished food intake, leading to a decreased energy intake, thus preventing an increase in weight-gain and attenuated glucose intolerance. Additionally, to my knowledge, this is the first study to demonstrate both a decrease in food consumption and energy intake, as well as an improved glucose tolerance when low-dose dietary capsaicin is administered as part of a healthy diet.

4.5 Hepatic dysfunction and plasma concentrations of triglycerides

The chronic release of free fatty acids and adipokines such as IL6, TNFα, MCP1, MIP2 and COX2 as well as a sustained increase in plasma triglyceride concentrations induces hepatic dysfunction and initiates oxidative stress by causing chronic inflammation, hepatocyte apoptosis, enhanced fat deposition and hepatic insulin resistance (Kang et al. 2010; Wei et al. 2013; Brown et al. 2015). This leads to non-alcoholic fatty liver disease, as well as other metabolic disease states such as type 2 diabetes, insulin resistance, dyslipidaemia and cardiovascular disease (Kang et al. 2010; Wei et al. 2013; Brown et al. 2015). Additionally, an atherogenic lipid profile is typically present in conjunction with non-alcoholic fatty liver disease, with increased concentrations of triglycerides, NEFA and total cholesterol (Panchal et al. 2012; Brown et al. 2015). Increased free fatty acids and inflammatory adipokines, which result in dyslipidaemia are evident in diet-induced obese subjects (Cohen & Fisher 2013). Although free fatty acids and inflammatory adipokines were not measured in this study, markers of dyslipidaemia, including triglycerides and NEFA, were increased in H-fed rats. While low-dose dietary capsaicin reduced plasma concentrations of total cholesterol independent of diet, H-fed rats did not demonstrate an increase outside of normal physiological parameters in plasma concentrations of total cholesterol (Mary et al. 2006). Furthermore, since rats do not exhibit a notable change in HDL and LDL concentrations, it is not clear if a decrease in

plasma HDL concentration was evident (Chen et al. 2014). Hence, the primary marker of dyslipidaemia used in this study was the increased concentrations of plasma triglycerides, as one of the five risk factors associated with metabolic syndrome (Harris 2013).

H-fed rats exhibited non-alcoholic fatty liver disease, shown by increased plasma liver damage enzymes, namely AST and ALT, increased inflammatory cell migration, steatosis and an augmented liver wet weight. The literature suggests that subjects with these parameters also possess hepatocytes with impaired plasmalemmal expression of cholesterol uptake receptors and reverse cholesterol transporters, thus leading to dyslipidaemia and steatosis within the liver (Kang et al. 2010; Ma et al. 2011; Wei et al. 2013; Li et al. 2014). Additionally, increased inflammatory adipokines and free fatty acid concentrations block insulin receptors within hepatocytes, thus contributing to dyslipidaemia and glucose intolerance (Kang et al. 2010; Wei et al. 2013; Borbély et al. 2015). Dietary capsaicin administration decreased plasma concentrations of adipokines, free fatty acids, triglycerides, total cholesterol and glucose (Kang et al. 2010; Wei et al. 2013). Capsaicin induced an increased expression of hepatocyte cholesterol uptake receptors and reverse cholesterol transporters, thus promoting cholesterol and triglyceride clearance, in addition to relieving steatosis within the liver (Kang et al. 2010; Wei et al. 2013). Although the exact mechanisms involved are yet to be explored and elucidated, my study supported the literature in demonstrating that low-dose dietary capsaicin attenuated dyslipidaemia, exemplified by an increase in plasma triglyceride concentration, and glucose intolerance, by improving liver structure and function. Liver structure and hepatocyte damage was improved by low-dose dietary capsaicin and was established by decreasing plasma concentrations of AST and ALT, as well as decreasing inflammatory cell infiltration, fibrosis and steatosis, which, in turn, decreased the wet weight of the liver. Hence low-dose dietary capsaicin treatment attenuated obesity-induced non-alcoholic fatty liver disease.

4.6 Cardiovascular health

The determinants of metabolic syndrome, that is obesity, dyslipidaemia, glucose intolerance and hypertension, lead to cardiovascular remodelling (Nikolopoulou & Kadoglou 2012). Additionally, inflammatory adipokines and free fatty acid release from abdominal fat pads are involved in cardiovascular remodelling (Pala et al. 2011). H-fed rats exhibited hypertension and cardiovascular remodelling, which was demonstrated by increased collagen deposition and inflammatory cell infiltration within the heart, thus leading to an increased myocardial wet weight, increased diastolic stiffness and an impaired response to acetylcholine, thus indicating endothelial damage. Low-dose dietary capsaicin treatment reversed hypertension, endothelial damage and the diastolic stiffness of the heart, as well as reduced inflammatory cell infiltration and collagen deposition within the heart, thus reducing the overall myocardial wet weight in H-fed rats. These findings support the literature in that low-dose capsaicin relieves hypertension and improves cardiovascular function (Yang et al. 2010; Xu et al. 2011; Robbins et al. 2013; Wang et al. 2014). Additionally, to my knowledge, this is the first study that has demonstrated an improved endothelial response and reduction in systolic blood pressure in the presence of a heathy diet.

The endothelial layer of the vasculature expresses TRPV1 channels (Inoue et al. 2006). Capsaicin activates TRPV1 in these cells and consequently increases cytosolic Ca^{2+}, which signals PKA to activate eNOS (Yang et al. 2010; Xu et al. 2011). The activation of eNOS results in nitric oxide release. Nitric oxide induces potent vascular smooth muscle relaxation, which, in turn, causes vasodilatation and a subsequent decrease in systolic blood pressure (Yang et al. 2010; Xu et al. 2011). Whilst the molecular mechanism as to how low-dose dietary capsaicin exerts its effects was not measured in this study, an improved response to acetylcholine-induced relaxation was observed. Acetylcholine binds to the muscarinic receptors on vascular smooth muscle cells, which subsequently increases nitric oxide production and induces relaxation in the endothelial layer of the vasculature (Tangsucharit et al. 2016). The impaired response of H-fed rats to acetylcholine

indicates endothelial dysfunction, whilst the improved response in low-dose dietary capsaicin treated rats demonstrates reversal of this damage and an increased sensitivity to nitric oxide. Hence, this supports the literature in how capsaicin induces its effects within the vasculature and reduces systolic blood pressure (Figure 18) (Yang et al. 2010; Xu et al. 2011).

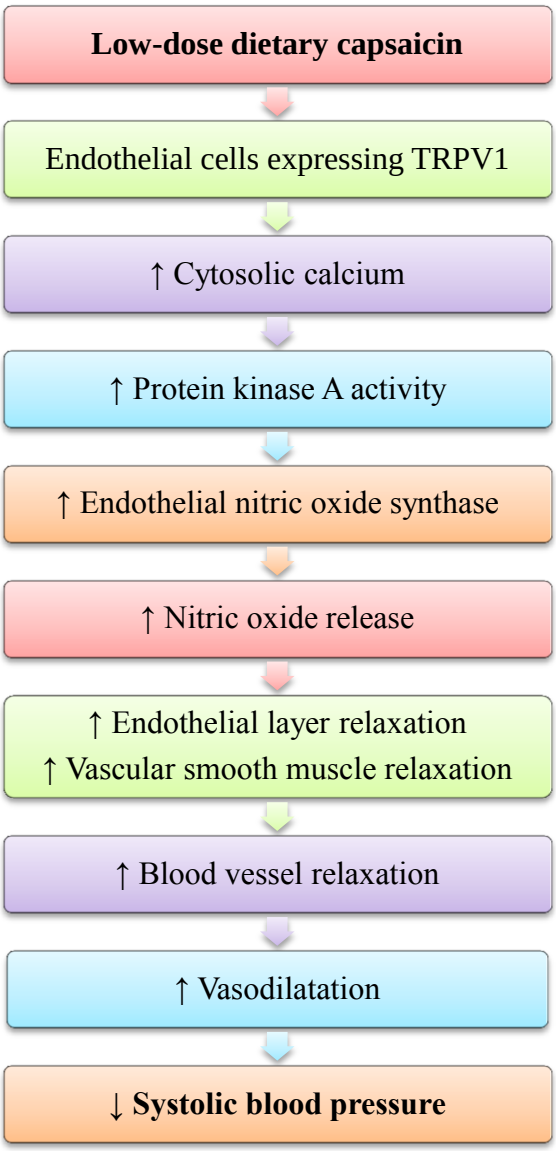


Figure 18: Proposed pathway of mechanisms by which low-dose dietary capsaicin relieves hypertension.

TRPV1-expressing afferents are intricately associated with the cardiovascular system and, therefore, capsaicin may exert its effects on the vasculature via an alternative mechanism (Tominaga et al. 1998; Inoue et al. 2006). Capsaicin, through the activation of TRPV1, induces the release of CGRP and substance P in afferents supplying the vasculature, via an increase in Ca^{2+} (Li

& Wang 2003; Zvara et al. 2005; Fattori et al. 2016). The increased release of these chemicals results in potent vasodilatation, thus decreasing pressure within the vasculature and, therefore, relieving hypertension (Li & Wang 2003; Zvara et al. 2005; Russell et al. 2014). Hence, the impact that these chemokines may have had on this study cannot be excluded. Additionally, low-dose capsaicin decreased renal pressure, increased glomerular filtration rate and increased water/sodium excretion, thus mediating renal function and, consequently, blood pressure (Li & Wang 2008). Hence, these mechanisms may assist in the regulation of blood pressure. Future studies could aim to induce impaired renal function in rats via a high-salt diet or measure renal function markers to investigate the long-term effects conveyed by low-dose dietary capsaicin on renal function and, subsequently, blood pressure.

H-fed rats displayed increased inflammatory cell infiltration and collagen deposition within the heart. Increased cardiac collagen deposition is a direct marker of cardiac hypertrophy (Wang et al. 2014). This was demonstrated by an increased wet weight of the left ventricle and septum. Inflammatory cell infiltration within the heart, contributes to the development of fibrosis, as their initial presence within the perivascular region of the heart recruits cardiac fibroblasts to produce collagen-1(Wang et al. 2014). This subsequently results in an increase in collagen synthesis and perivascular fibrosis. Inflammatory cells continue to further infiltrate the interstitial space, thus triggering the increased synthesis of collagen and its deposition, resulting in fibrosis extending between myocytes, which, in turn, results in interstitial fibrosis and an insulating effect on myocyte bundles (Krenning et al. 2010; Wang et al. 2016). This ultimately leads to altered electrophysiology and an increase in diastolic stiffness, which can be directly measured by the Langendorff heart (Krenning et al. 2010). Diastolic stiffness in H-fed rats was increased when compared to C-fed rats.

Low-dose dietary capsaicin treatment reduced cardiac collagen deposition and inflammatory cell infiltration and, consequently, reduced the wet weight of the left ventricle and diastolic stiffness evident in H-fed rats. TRPV1 is expressed in afferent fibres innervating the heart, as well as in

myocytes and within cells of the endocardium (Zvara et al. 2005; Torres-Narváez et al. 2012). Capsaicin-induced activation of TRPV1 reduced fibrosis, inflammation and the diastolic stiffness in the heart (Zvara et al. 2005; Torres-Narváez et al. 2012; Wang et al. 2014). These capsaicin-induced effects are not displayed in TRPV1 knockout mice (Wang et al. 2014). Hence, TRPV1 mediates the effects of capsaicin within heart.

How TRPV1 activation by capsaicin conveys its cardio-protectant effects on the heart are complex and varied. The influx of cytosolic Ca^{2+} within endothelial cells initiates a chain of events that induce the upregulation of an array of proteins and chemokines (Zvara et al. 2005; McCarty et al. 2015). Of particular importance is the rise in eNOS expression and subsequent release of nitric oxide (Zvara et al. 2005; Torres-Narváez et al. 2012). The endocardium of the heart is lined with endothelial cells that are biologically akin to endothelial cells lining the vasculature (Hall 2015). Therefore, the production of nitric oxide by eNOS within these cells probably involves the same molecular pathway than that of the endothelial cells of the vasculature. Providing evidence to this notion are studies that have demonstrated a reduced expression of eNOS and nitric oxide production within both the arteries and the heart of rats treated with the known TRPV1 antagonist capsazepine (Torres-Narváez et al. 2012).

Low-dose capsaicin increases the concentrations of CGRP within the heart (Zvara et al. 2005). Although increased CGRP concentrations have been correlated with reductions in cardiac inflammation (both cells and chemokines, such as IL6), fibrosis and hypertension, as well as improving inotropic and chronotropic responses, the exact mechanisms as to how it conveys its effects remain elusive (Muddhrry et al. 1988; Russell et al. 2014; Zheng et al. 2015). Furthermore, CGRP-containing neurons innervate the atrioventricular and sinoatrial nodes, as well as the myocardium and coronary arteries (Muddhrry et al. 1988; Ieda et al. 2006). Hence, it is hypothesised that CGRP, released by capsaicin-induced TRPV1 activation, exerts its effects in a similar manner as on the vasculature, which is ultimately to increase relaxation within myocytes

(Russell et al. 2014; Zheng et al. 2015). Additionally, CGRP release in response to low-dose dietary capsaicin at the cardiac electrical nodes may improve cardiac efficiency. Since CGRP typically acts to relax muscle by decreasing electrical excitability, it may improve cardiac efficiency by reducing the heart rate, which increases the fill-time within the cardiac chambers (atria and ventricles), resulting in a greater stroke volume and, therefore, cardiac output (Russell et al. 2014; Hall 2015). Whilst this may appear as a plausible hypothesis as to how low-dose dietary capsaicin may ultimately induce its cardio-protective effects, further studies would need to be performed in support of this. However, what is clear from this study is that low-dose dietary capsaicin treatment reverses the damage conferred by a hypercaloric diet, thus decreasing the likelihood of cardiovascular disease.

4.7 Gastrointestinal function

Rats treated with low-dose dietary capsaicin demonstrated improved contractility within the ileum. It is accepted that low-dose dietary capsaicin acts as a gastro-protective molecule and improves gastrointestinal function (Gy et al. 2001; Mózsik et al. 2005; Mózsik et al. 2009a). However, there is a gap in the literature with respect to isolated intestinal-organ baths in various species treated with low-dose capsaicin. This could be due to further advances in molecular testing or a large focus on human gastrointestinal health where it is not possible to perform isolated organ baths. Either way, the mechanisms by which low-dose dietary capsaicin acts a gastro-protectant remain elusive and somewhat controversial. The current accepted theory is that dietary capsaicin induces the release of CGRP via the activation of the TRPV1 receptor (Abdel-Salam 2014). CGRP is expressed throughout the entire central and peripheral nervous systems, where its function differs depending on where it is synthesised (Abdel-Salam 2014; Russell et al. 2014). Extrinsic spinal afferents and vagal afferents activated by low-dose dietary capsaicin, synapse with their cell bodies in either the dorsal root ganglion or the nodose ganglia respectively (Holzer 2007; Evangelista 2009). These extrinsic nerve terminals only expressed α-CGRP and innervated gastrointestinal smooth muscle

layers, the myenteric plexus and the submucosal plexus (Holzer 2007; Evangelista 2009). Intrinsic enteric neurons of the enteric nervous system expressed β-CGRP and were localised within the myenteric plexus (Holzer 2007; Evangelista 2009; Abdel-Salam 2014). α-CGRP, in humans, directly exerted its actions on the muscle layers throughout the intestine and caused relaxation (Holzer 2007; Evangelista 2009; Russell et al. 2014). β-CGRP expressed by the enteric nervous system appears to indirectly stimulate neuronal receptors to release neurotransmitters, which, in turn, convey different effects on intestinal motility depending on the species (Holzer 2007; Evangelista 2009; Russell et al. 2014). For example, in the ileum of a guinea pig and various intestinal segments in other species, β-CGRP induced the release of acetylcholine, which subsequently activated muscarinic acetylcholine receptors in the ileum to induce a contraction (Someya et al. 2003; Barthó et al. 2004; Matsumoto et al. 2009). Hence, this may explain the improved ileum contractility observed in low-dose dietary capsaicin treated rats.

The contrary occurs in humans, where it is accepted that both isoforms of CGRP induce gastrointestinal myocytes to relax (Holzer 2007; Evangelista 2009). Additionally, larger animals such as a human or pig, digest macronutrients, in particular fibre, to a greater extent than smaller animals such as a rat or guinea pig (Nyman et al. 1986). Since smaller animals, such as a rat, require more energy per unit of body-weight, the transit time of foodstuffs throughout the gut is shorter and fibre utilisation is lower (Nyman et al. 1986). Hence, it is unclear if the findings of increased contractility within the ileum of a rat model can be clearly correlated to humans. Hence, future studies could use modern molecular techniques to determine if low-dose dietary capsaicin initiates an increased CGRP expression throughout the gut and correlate these findings with changes in human gastrointestinal tract function and health.

Chapter 5: Conclusions

The primary conclusions of this study are that low-dose dietary capsaicin produced added health benefits to rats on a healthy diet and attenuated the signs of metabolic syndrome in diet-induced obese rats. Support for the first conclusion is that low-dose dietary capsaicin improved some physiological parameters in C-fed rats. Low-dose dietary capsaicin reduced abdominal adiposity, abdominal circumference and body weight in C-fed rats. Additionally, it improved glucose tolerance and utilisation within C-fed rats, reduced food intake and energy consumption, together with improved endothelial function and lowered systolic blood pressure. To my knowledge, this is the first study to demonstrate improved physiological parameters when low-dose dietary capsaicin is administered in the presence of a healthy diet.

The second conclusion is supported by my findings that low-dose dietary capsaicin reduced central obesity, reversed glucose intolerance, decreased plasma concentrations of triglycerides and reduced hypertension, which are the physiological and biochemical changes occurring in metabolic syndrome, as well as alleviated non-alcoholic fatty liver disease and cardiovascular dysfunction. Therefore, both hypotheses of this study were accepted.

Overall, this study demonstrated that low-dose dietary capsaicin could be used as an inexpensive and novel therapeutic agent to attenuate obesity and metabolic syndrome. The literature cited describes the effects that low-dose dietary capsaicin may have on the individual determinants of metabolic syndrome and the complex changes it induces molecularly and, therefore, physiologically. However, to my knowledge, this is the first study since the discovery of TRPV1 to focus on the changes low-dose dietary capsaicin induces on multiple physiological and biochemical parameters and targets the pathologies that drive metabolic syndrome throughout the whole body. Finally, this study is the first of its kind in Australia, where diet-induced obesity and metabolic syndrome are prevalent, to explore the effects low-dose dietary capsaicin conveys in a diet-induced obesity model. Hence, this study provides the foundations for future endeavours to further critically analyse the use of low-dose dietary capsaicin in the prevention and treatment of metabolic syndrome, obesity and its associated complications.

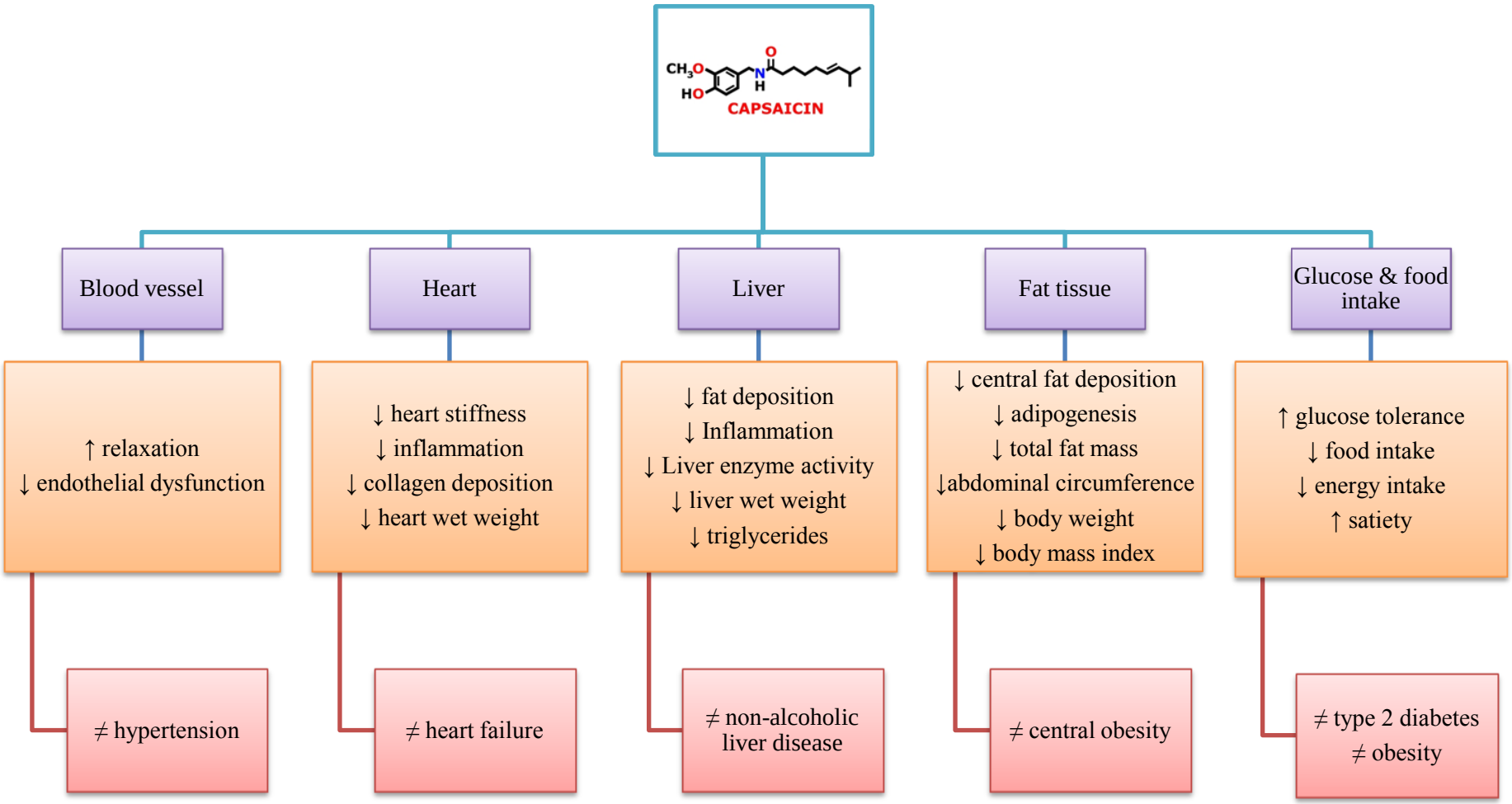


Figure 19: Project summary of the responses to low-dose dietary capsaicin.

Chapter 6: Future directions

This study developed a simple and inexpensive extraction method of obtaining a relatively high yield of capsaicin. Hence, future studies that use the methods outlined here to extract capsaicin may wish to determine the economic cost and practicality of developing this on a larger scale for industrial application. Future studies may also wish to use more piquant chillies than the ones utilised in this procedure, in order to determine if a greater yield of capsaicin could be generated.

Because low-dose dietary capsaicin attenuated the cardiometabolic parameters in H-fed rats and produced health benefits in C-fed rats, future studies could determine and quantify the expression of different proteins, such as TRPV1, chemokines, including CGRP and GLP1, and inflammatory markers such as IL6 and TNFα, that are evident systemically and within specific tissues. Large quantities of tissue samples were collected, stored and frozen at -80ºC and fixed in formalin from the rats sacrificed in this protocol. Using these samples would assist in providing evidence of the mechanisms involved in low-dose dietary capsaicin treatment. Providing evidence of the molecular pathways and linking it to the literature and the outcomes generated from this study would assist in providing a foundation in progressing capsaicin usage into future human trials.

Since low-dose dietary capsaicin reduced cardiac and hepatic inflammatory cell infiltration, thus improving cardiac structure and function, future studies could aim at exploring the use of low-dose dietary capsaicin as an anti-inflammatory compound in other inflammatory diseases, such as inflammatory bowel disease. General histology of the gastrointestinal tract, as well as mediators of inflammation associated with inflammatory bowel disease could be measured and assist in determining capsaicin's role as a gastro-protective molecule. Adding more complexity to pathways and responses that low-dose dietary capsaicin may elicit is the progression made in understanding of the human microbiome and how microbes in this complex ecosystem influence an individual's physiology and behaviours. Progress in this area is still in its early stages. However, progress with respect to how microbes within the human gastrointestinal tract influence an individual's

physiology and behaviour has already come so far as to redescribe and re-term the gut-brain axis as the gut-brain-microbiota axis (Moran & Shanahan 2014). Given the intricate interactions between capsaicin, TRPV1 and the nervous system, there is no doubt that future studies will need to explore the possible influences that low-dose dietary capsaicin may have on the gut microbiota and, in turn, on an individual's physiology and behaviour. Faecal samples from the rats sacrificed in this protocol were collected and have been frozen for future investigations.

Legs of the rats sacrificed in this protocol have been stored in formalin. Since, low-dose dietary capsaicin has anti-inflammatory properties, its use in osteoarthritis may be beneficial to explore. Concentrations of general inflammatory markers, histological staining of the knee joints to determine articular cartilage degradation, osteocyte counts and subchondral bone changes using micro-computerised tomography could be used to determine the possible anti-inflammatory role low-dose dietary capsaicin conveys in relation to osteoarthritis, as our research group has recently reported with lauric acid in H rats (Sekar et al. 2017).

One of the major mechanisms of regulating systolic blood pressure is kidney function. Kidney function was not measured in this study. Hence, improved renal function could not be excluded from assisting in the regulation of blood pressure. Future studies could aim to induce impaired renal function in rats via a high-salt diet or measure renal function markers, such as uric acid, urea, creatinine and estimated glomerular filtration rate, to investigate the long-term effects conveyed by low-dose dietary capsaicin on renal function and, subsequently, blood pressure.

Diet-induced obesity conveys an increased risk of developing a range of diseases, including cancer. The H diet induced obesity and the signs of metabolic syndrome and, therefore, may act as a model to test various carcinomas, such as prostate cancer. A further study using the tissues from the rats sacrificed in this protocol aims to determine the potential anti-cancer properties that low-dose dietary capsaicin may convey, as in a 2016 project using curcumin with Amanda Dieckmann, Dr Sunil Panchal and Dr Eliza Whiteside. Since, low-dose dietary capsaicin attenuated the

cardiometabolic signs of metabolic syndrome, capsaicin should reduce the expression of markers related to the development of colon cancer, and therefore, suggest a role as a supplement with anti-carcinogenic properties.

This study supports the findings of other studies in identifying the potential health benefits of low-dose dietary capsaicin and the potential to translate these findings to humans by undertaking human clinical trials. Hence, future studies using dietary capsaicin should aim to define a low-dose that would be effective in combating the pathophysiological changes of metabolic syndrome. This study suggests that approximately 97.2 mg/day or 1.62 mg/kg of body weight/day for a 60 kg human would impart potential health benefits. However, collaborations with other researchers in relation to the conduct of human clinical trials may be of benefit to review the dose needed to elicit the effects of low-dose dietary capsaicin and protocol requirements. Human clinical trials should aim to measure central obesity markers, blood pressure, glucose tolerance, plasma lipid profiles, in particular HDL and triglycerides, liver function markers, renal function markers, inflammatory markers and various other chemical messengers, such as CGRP and GLP1 concentrations to ascertain the effectiveness of low-dose dietary capsaicin in attenuating these physiological changes. Additionally, the differences in dyslipidaemia, in particular HDL and LDL and, therefore, atherosclerosis are more pronounced in humans. Hence, measuring these markers in humans would further demonstrate low-dose capsaicin's efficacy in treating cardiovascular disease, which is still the number one cause of mortality globally, and the complex haemodynamic properties that are evident within this system.

Different dosages of capsaicin have been established to be efficacious in providing pain relief by causing neuronal desensitisation. This effect is described in 1.4.5 TRPV1 desensitisation and modulation. Hence, it is unknown if prolonged use of oral low-dose dietary capsaicin would induce desensitisation in the gastrointestinal tract, heart or liver and, therefore, render capsaicin treatment ineffective. Therefore, future studies investigating the prolonged oral use of low-dose dietary

capsaicin administration would be of benefit. Long-term studies that investigate chronic low-dose dietary capsaicin use would be able to provide insight into the possible adverse effects that may occur. Hence, there still remains an extensive amount of research to be performed on the potential health benefits of low-dose dietary capsaicin.

References

Abbott, CR, Monteiro, M, Small, CJ, Sajedi, A, Smith, KL, Parkinson, JRC, Ghatei, MA & Bloom, SR 2005, 'The inhibitory effects of peripheral administration of peptide YY3–36 and glucagon-like peptide-1 on food intake are attenuated by ablation of the vagal–brainstem–hypothalamic pathway', *Brain Research*, vol. 1044, no. 1, pp. 127-31.

Abdel-Salam, OM 2014, *Capsaicin as a therapeutic molecule*, Springer, Basel.

Adam, TC & Westerterp-Plantenga, MS 2005, 'Glucagon-like peptide-1 release and satiety after a nutrient challenge in normal-weight and obese subjects', *British Journal of Nutrition*, vol. 93, no. 06, pp. 845-51.

Aekplakorn, W, Hogan, MC, Chongsuvivatwong, V, Tatsanavivat, P, Chariyalertsak, S, Boonthum, A, Tiptaradol, S & Lim, SS 2007, 'Trends in Obesity and Associations with Education and Urban or Rural Residence in Thailand', *Obesity*, vol. 15, no. 12, pp. 3113-21.

Ambrosino, P, Soldovieri, MV, Russo, C & Taglialatela, M 2013, 'Activation and desensitization of TRPV1 channels in sensory neurons by the PPARα agonist palmitoylethanolamide', *British Journal of Pharmacology*, vol. 168, no. 6, pp. 1430-44.

Ashwini, D, Sree, MGU, Ajitha, MA & Rao, VUM 2015a, 'Extraction of capsaicin from Capsicum frutescens. L and its estimation by RP-HPLC method', *World Journal of Pharmacy and Pharmaceutical Sciences*, vol. 4, no. 9, pp. 839-48.

Bae, C, Anselmi, C, Kalia, J, Jara-Oseguera, A, Schwieters, CD, Krepkiy, D, Lee, CW, Kim, E-H, Kim, JI & Faraldo-Gómez, JD 2016, 'Structural insights into the mechanism of activation of the TRPV1 channel by a membrane-bound tarantula toxin', *Life*, vol. 5.

Bang, S, Yoo, S, Oh, U & Hwang, SW 2010, 'Endogenous lipid-derived ligands for sensory TRP ion channels and their pain modulation', *Archives of Pharmacalogical Research*, vol. 33, no. 10, pp. 1509-20.

Barthó, L, Benkó, R, Patacchini, R, Pethö, G, Holzer-Petsche, U, Holzer, P, Lázár, Z, Undi, S, Illényi, L, Antal, A & Horváth, Ö-P 2004, 'Effects of capsaicin on visceral smooth muscle: a valuable tool for sensory neurotransmitter identification', *European Journal of Pharmacology*, vol. 500, no. 1–3, pp. 143-57.

Bauer, PV, Hamr, SC & Duca, FA 2016, 'Regulation of energy balance by a gut–brain axis and involvement of the gut microbiota', *Cellular and Molecular Life Sciences*, vol. 73, no. 4, pp. 737-55.

Berthoud, H-R, Kressel, M, Raybould, HE & Neuhuber, WL 1995, 'Vagal sensors in the rat duodenal mucosa: distribution and structure as revealed by in vivo DiI-tracing', *Anatomy and Embryology*, vol. 191, no. 3, pp. 203-12.

Bertini, I, McGreevy, KS & Parigi, G 2012, *NMR of biomolecules towards mechanistic systems biology*, Wiley-VCH, Weinheim, pp. 33-170.

Betts, TA 1999, 'Pungency Quantitation of Hot Pepper Sauces Using HPLC', *Journal of Chemical Education*, vol. 76, no. 2, p. 240.

Bhaswant, M, Poudyal, H, Mathai, M, Ward, L, Mouatt, P & Brown, L 2015a, 'Green and Black Cardamom in a Diet-Induced Rat Model of Metabolic Syndrome', *Nutrients*, vol. 7, no. 9, p. 5360.

Bhaswant, M, Fanning, K, Netzel, M, Mathai, ML, Panchal, SK & Brown, L 2015b, 'Cyanidin 3-glucoside improves diet-induced metabolic syndrome in rats', *Pharmacological Research*, vol. 102, pp. 208-17.

Bielefeldt, K & Davis, BM 2008, 'Differential effects of ASIC3 and TRPV1 deletion on gastroesophageal sensation in mice', *American Journal of Physiology - Gastrointestinal and Liver Physiology*, vol. 294, no. 1, pp. G130-G8.

Blennerhassett, MG, Vignjevic, P, Vermillion, DL & Collins, SM 1992, 'Inflammation causes hyperplasia and hypertrophy in smooth muscle of rat small intestine', *American Journal of Physiology - Gastrointestinal and Liver Physiology*, vol. 262, no. 6, pp. G1041-G6.

Borbély, É, Botz, B, Bölcskei, K, Kenyér, T, Kereskai, L, Kiss, T, Szolcsányi, J, Pintér, E, Csepregi, JZ & Mócsai, A 2015, 'Capsaicin-sensitive sensory nerves exert complex regulatory functions in the serum-transfer mouse model of autoimmune arthritis', *Brain, Behavior, and Immunity*, vol. 45, pp. 50-9.

Brown, L, Poudyal, H & Panchal, SK 2015, 'Functional foods as potential therapeutic options for metabolic syndrome', *Obesity Reviews*, vol. 16, no. 11, pp. 914-41.

Buhmann, H, le Roux, CW & Bueter, M 2014, 'The gut–brain axis in obesity', *Best Practice & Research Clinical Gastroenterology*, vol. 28, no. 4, pp. 559-71.

Cao, E, Liao, M, Cheng, Y & Julius, D 2013a, 'TRPV1 structures in distinct conformations reveal mechanisms of activation', *Nature*, vol. 504, no. 7478, pp. 113-8.

Cao, E, Cordero-Morales, JF, Liu, B, Qin, F & Julius, D 2013b, 'TRPV1 channels are intrinsically heat sensitive and negatively regulated by phosphoinositide lipids', *Neuron*, vol. 77, no. 4, pp. 667-79.

Caterina, MJ, Schumacher, MA, Tominaga, M, Rosen, TA, Levine, JD & Julius, D 1997, 'The capsaicin receptor: a heat-activated ion channel in the pain pathway', *Nature*, vol. 389, no. 6653, pp. 816-24.

Chen, J, Li, L, Li, Y, Liang, X, Sun, Q, Yu, H, Zhong, J, Ni, Y, Chen, J, Zhao, Z, Gao, P, Wang, B, Liu, D, Zhu, Z & Yan, Z 2015, 'Activation of TRPV1 channel by dietary capsaicin improves visceral fat remodeling through connexin43-mediated Ca2+ Influx', *Cardiovascular Diabetology*, vol. 14, no. 1, p. 22.

Chen, Z, Li, S, Zhao, W, Chen, X & Wang, X 2014, 'Protective effect of co-administration of rosuvastatin and probucol on atherosclerosis in rats', *Canadian Journal of Physiology and Pharmacology*, vol. 92, no. 10, pp. 797-803.

Cohen, DE & Fisher, EA 2013, 'Lipoprotein Metabolism, Dyslipidemia and Nonalcoholic Fatty Liver Disease', *Seminars in Liver Disease*, vol. 33, no. 4, pp. 380-8.

Cone, R, Cowley, M, Butler, A, Fan, W, Marks, D & Low, M 2001, 'The arcuate nucleus as a conduit for diverse signals relevant to energy homeostasis', *International Journal of Obesity*, vol. 25, no. S5, p. S63.

Cooke, AR & Clark, ED 1976, 'Effect of first part of duodenum on gastric emptying in dogs: response to acid, fat, glucose, and neural blockade', *Gastroenterology*, vol. 70, no. 4, pp. 550-5.

Cowley, MA, Smart, JL, Rubinstein, M, Cerdan, MG, Diano, S, Horvath, TL, Cone, RD & Low, MJ 2001, 'Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus', *Nature*, vol. 411, no. 6836, pp. 480-4.

Craig, AD 1996, 'Chapter 13 An ascending general homeostatic afferent pathway originating in lamina I', *Progress in Brain Research*, vol. 107, pp. 225-42.

Daly, DM, Park, SJ, Valinsky, WC & Beyak, MJ 2011, 'Impaired intestinal afferent nerve satiety signalling and vagal afferent excitability in diet induced obesity in the mouse', *The Journal of Physiology*, vol. 589, no. 11, pp. 2857-70.

de Souza, RJ, Bray, GA, Carey, VJ, Hall, KD, LeBoff, MS, Loria, CM, Laranjo, NM, Sacks, FM & Smith, SR 2012, 'Effects of 4 weight-loss diets differing in fat, protein, and carbohydrate on fat mass, lean mass, visceral adipose tissue, and hepatic fat: results from the POUNDS LOST trial', *The American Journal of Clinical Nutrition*, vol. 95, no. 3, pp. 614-25.

DeFelice, SL 1995, 'The nutraceutical revolution: its impact on food industry R&D', *Trends in Food Science & Technology*, vol. 6, no. 2, pp. 59-61.

Desborough, MJR & Keeling, DM 2017, 'The aspirin story – from willow to wonder drug', *British Journal of Haematology*.

Devesa, I, Ferrándiz-Huertas, C, Mathivanan, S, Wolf, C, Luján, R, Changeux, J-P & Ferrer-Montiel, A 2014, 'αCGRP is essential for algesic exocytotic mobilization of TRPV1 channels in peptidergic nociceptors', *Proceedings of the National Academy of Sciences*, vol. 111, no. 51, pp. 18345-50.

Dezhdar, T, Moshourab, RA, Fründ, I, Lewin, GR & Schmuker, M 2015, 'A Probabilistic Model for Estimating the Depth and Threshold Temperature of C-fiber Nociceptors', *Scientific Reports*, vol. 5.

Dhaka, A, Uzzell, V, Dubin, AE, Mathur, J, Petrus, M, Bandell, M & Patapoutian, A 2009, 'TRPV1 is activated by both acidic and basic pH', *The Journal of Neuroscience*, vol. 29, no. 1, pp. 153-8.

Diepvens, K, Westerterp, KR & Westerterp-Plantenga, MS 2007, 'Obesity and thermogenesis related to the consumption of caffeine, ephedrine, capsaicin, and green tea', *American Journal of Physiology-Regulatory, Integrative and Comparative physiology*, vol. 292, no. 1, pp. R77-R85.

Diwan, V, Poudyal, H & Brown, L 2013, 'Piperine Attenuates Cardiovascular, Liver and Metabolic Changes in High Carbohydrate, High Fat-Fed Rats', *Cell Biochemistry and Biophysics*, vol. 67, no. 2, pp. 297-304.

Dockray, GJ 2013, 'Enteroendocrine cell signalling via the vagus nerve', *Current Opinion in Pharmacology*, vol. 13, no. 6, pp. 954-8.

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*, vol. 342, no. 3, pp. 357-61.

Dryden, S, Pickavance, L, Frankish, HM & Williams, G 1995, 'Increased neuropeptide Y secretion in the hypothalamic paraventricular nucleus of obese (fa/fa) Zucker rats', *Brain Research*, vol. 690, no. 2, pp. 185-8.

Elliott, RM, Morgan, LM, Tredger, JA, Deacon, S, Wright, J & Marks, V 1993, 'Glucagon-like peptide-1(7–36)amide and glucose-dependent insulinotropic polypeptide secretion in response to nutrient ingestion in man: acute post-prandial and 24-h secretion patterns', *Journal of Endocrinology*, vol. 138, no. 1, pp. 159-66.

Enriori, PJ, Evans, AE, Sinnayah, P, Jobst, EE, Tonelli-Lemos, L, Billes, SK, Glavas, MM, Grayson, BE, Perello, M, Nillni, EA, Grove, KL & Cowley, MA 2007, 'Diet-Induced Obesity Causes Severe but Reversible Leptin Resistance in Arcuate Melanocortin Neurons', *Cell Metabolism*, vol. 5, no. 3, pp. 181-94.

Evangelista, S 2009, 'Role of Calcitonin Gene-Related Peptide in Gastric Mucosal Defence and Healing', *Current Pharmaceutical Design*, vol. 15, no. 30, pp. 3571-6.

Fattori, V, Hohmann, MS, Rossaneis, AC, Pinho-Ribeiro, FA & Verri, WA 2016, 'Capsaicin: Current Understanding of Its Mechanisms and Therapy of Pain and Other Pre-Clinical and Clinical Uses', *Molecules*, vol. 21, no. 7, p. 844.

Gamse, R 1982, 'Capsaicin and nociception in the rat and mouse. Possible role of substance P', *Naunyn-Schmiedeberg's Archives of Pharmacology*, vol. 320, no. 3, pp. 205-16.

Glinsukon, T, Stitmunnaithum, V, Toskulkao, C, Buranawuti, T & Tangkrisanavinont, V 1980, 'Acute toxicity of capsaicin in several animal species', *Toxicon*, vol. 18, no. 2, pp. 215-20.

Goci, E, Haloçi, E, Vide, K & Malaj, L 2014, 'Application and comparison of three different extraction methods of capsaicin from capsicum fruits', *Albanian Journal of Pharmaceutical Sciences*, vol. 1, no. 1, pp. 16-9.

Gottlieb, HE, Kotlyar, V & Nudelman, A 1997, 'NMR chemical shifts of common laboratory solvents as trace impurities', *The Journal of Organic Chemistry*, vol. 62, no. 21, pp. 7512-5.

Gribble, FM & Reimann, F 2016, 'Enteroendocrine Cells: Chemosensors in the Intestinal Epithelium', *Annual Review of Physiology*, vol. 78, no. 1, pp. 277-99.

Grima, M & Dixon, J 2013, 'Obesity Recommendations for management in general practice and beyond', *Australian Family Physician*, vol. 42, pp. 532-41.

Guido, PC, Ribas, A, Gaioli, M, Quattrone, F & Macchi, A 2015, 'The state of the integrative medicine in Latin America: The long road to include complementary, natural, and traditional practices in formal health systems', *European Journal of Integrative Medicine*, vol. 7, no. 1, pp. 5-12.

Gy, M, Vincze, Á & Szolcsányi, J 2001, 'Four responses of capsaicin sensitive primary afferent neurons to capsaicin and its analog. Gastric acid secretion, gastric mucosal damage and protection', *Journal of Gastroenterology & Hepatology*, vol. 16, pp. 193-7.

Hakim, MA, Jiang, W, Luo, L, Li, B, Yang, S, Song, Y & Lai, R 2015, 'Scorpion toxin, BmP01, induces pain by targeting TRPV1 channel', *Toxins*, vol. 7, no. 9, pp. 3671-87.

Halford, JCG & Harrold, JA 2012, '5-HT2C Receptor Agonists and the Control of Appetite', in H-G Joost (ed.), *Appetite Control*, Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 349-56.

Hall, JE 2015, *Guyton and Hall Textbook of Medical Physiology*, 13 edn, Elsevier Health Sciences, Saint Louis, pp. 97-102, 179-84, 203-12, 227-41, 607-30, 787-90, 887-900.

Handwerker, H 2008, 'What Is a Polymodal Nociceptor?', *The Journal of Pain*, vol. 9, no. 4, pp. 309-10.

Hanson, SM, Newstead, S, Swartz, KJ & Sansom, MS 2015, 'Capsaicin interaction with TRPV1 channels in a lipid bilayer: molecular dynamics simulation', *Biophysical Journal*, vol. 108, no. 6, pp. 1425-34.

Harris, M 2013, 'The metabolic syndrome', *Australian Family Physician*, vol. 42, pp. 524-7.

Hayes, AJ, Lung, TWC, Bauman, A & Howard, K 2017, 'Modelling obesity trends in Australia: unravelling the past and predicting the future', *International Journal of Obesity*, vol. 41, no. 1, pp. 178-85.

Hayes, P, Meadows, HJ, Gunthorpe, MJ, Harries, MH, Duckworth, DM, Cairns, W, Harrison, DC, Clarke, CE, Ellington, K, Prinjha, RK, Barton, AJ, Medhurst, AD, Smith, GD, Topp, S, Murdock, P, Sanger, GJ, Terrett, J, Jenkins, O, Benham, CD, Randall, AD, Gloger, IS & Davis, JB 2000, 'Cloning and functional expression of a human orthologue of rat vanilloid receptor-1', *Pain*, vol. 88, no. 2, pp. 205-15.

Ho, KW, Ward, NJ & Calkins, DJ 2012, 'TRPV1: a stress response protein in the central nervous system', *American journal of Neurodegenerative Disease*, vol. 1, no. 1, p. 1.

Holzer, P 2007, 'Role of visceral afferent neurons in mucosal inflammation and defense', *Current Opinion in Pharmacology*, vol. 7, no. 6, pp. 563-9.

Hu, D-E, Easton, AS & Fraser, PA 2005, 'TRPV1 activation results in disruption of the blood–brain barrier in the rat', *British Journal of Pharmacology*, vol. 146, no. 4, pp. 576-84.

Huang, J, Mabury, SA & Sagebiel, JC 2000, 'Hot chili peppers: Extraction, cleanup, and measurement of capsaicin', *Journal of Chemical Education*, vol. 77, no. 12, p. 1630.

Hudson, ASR, Kunstetter, AC, Damasceno, WC & Wanner, SP 2016, 'Involvement of the TRPV1 channel in the modulation of spontaneous locomotor activity, physical performance and physical exercise-induced physiological responses', *Brazilian Journal of Medical and Biological Research*, vol. 49, no. 6, p. e5183.

Iannetti, GD, Baumgärtner, U, Tracey, I, Treede, R-D & Magerl, W 2013, 'Pinprick-evoked brain potentials: a novel tool to assess central sensitization of nociceptive pathways in humans', *Journal of Neurophysiology*, vol. 110, no. 5, pp. 1107-16.

Ieda, M, Kanazawa, H, Ieda, Y, Kimura, K, Matsumura, K, Tomita, Y, Yagi, T, Onizuka, T, Shimoji, K, Ogawa, S, Makino, S, Sano, M & Fukuda, K 2006, 'Nerve Growth Factor Is Critical for Cardiac Sensory Innervation and Rescues Neuropathy in Diabetic Hearts', *Circulation*, vol. 114, no. 22, pp. 2351-63.

Inoue, R, Jensen, LJ, Shi, J, Morita, H, Nishida, M, Honda, A & Ito, Y 2006, 'Transient receptor potential channels in cardiovascular function and disease', *Circulation Research*, vol. 99, no. 2, pp. 119-31.

Ishibashi, S, Goldstein, JL, Brown, MS, Herz, J & Burns, DK 1994, 'Massive xanthomatosis and atherosclerosis in cholesterol-fed low density lipoprotein receptor-negative mice', *Journal of Clinical Investigation*, vol. 93, no. 5, pp. 1885-93.

Jacobsen, NE 2016, *NMR Data Interpretation Explained*, John Wiley & Sons, Incorporated, Somerset, UNITED STATES, pp. 17-50.

Janssens, PL, Hursel, R & Westerterp-Plantenga, MS 2014, 'Capsaicin increases sensation of fullness in energy balance, and decreases desire to eat after dinner in negative energy balance', *Appetite*, vol. 77, pp. 46-51.

Jung, J, Shin, JS, Lee, S-Y, Hwang, SW, Koo, J, Cho, H & Oh, U 2004, 'Phosphorylation of vanilloid receptor 1 by Ca2+/calmodulin-dependent kinase II regulates its vanilloid binding', *Journal of Biological Chemistry*, vol. 279, no. 8, pp. 7048-54.

Kang, C, Zhang, Y, Zhu, X, Liu, K, Wang, X, Chen, M, Wang, J, Chen, H, Hui, S, Huang, L, Zhang, Q, Zhu, J, Wang, B & Mi, M 2016, 'Healthy Subjects Differentially Respond to Dietary Capsaicin Correlating with Specific Gut Enterotypes', *The Journal of Clinical Endocrinology & Metabolism*, vol. 101, no. 12, pp. 4681-9.

Kang, JH, Tsuyoshi, G, Han, IS, Kawada, T, Kim, YM & Yu, R 2010, 'Dietary capsaicin reduces obesity- induced insulin resistance and hepatic steatosis in obese mice fed a high- fat diet', *Obesity*, vol. 18, no. 4, pp. 780-7.

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*, vol. 73, no. 12, pp. 2690-7.

Kiyan, Y, Tkachuk, S, Hilfiker-Kleiner, D, Haller, H, Fuhrman, B & Dumler, I 2014, 'oxLDL induces inflammatory responses in vascular smooth muscle cells via urokinase receptor association with CD36 and TLR4', *Journal of Molecular and Cellular Cardiology*, vol. 66, pp. 72-82.

Koleva Gudeva, L, Maksimova, V, Serafimovska Darkovska, M, Gulaboski, R & Janevik-Ivanovska, E 2013, 'The effect of different methods of extractions of capsaicin on its content in the capsicum oleoresins', *Scientific Works: Food Science, Engineering and Technology 2013*, vol. 60, pp. 917-22.

Kozak, LP & Anunciado-Koza, R 2008, 'UCP1: its involvement and utility in obesity', *International Journal of Obesity (2005)*, vol. 32, no. Suppl 7, pp. S32-S8.

Kraft, KH, Brown, CH, Nabhan, GP, Luedeling, E, Ruiz, JdJL, d'Eeckenbrugge, GC, Hijmans, RJ & Gepts, P 2014, 'Multiple lines of evidence for the origin of domesticated chili pepper, Capsicum annuum, in Mexico', *Proceedings of the National Academy of Sciences*, vol. 111, no. 17, pp. 6165-70.

Krenning, G, Zeisberg, EM & Kalluri, R 2010, 'The origin of fibroblasts and mechanism of cardiac fibrosis', *Journal of Cellular Physiology*, vol. 225, no. 3, pp. 631-7.

Kumar, R, Hazan, A, Basu, A, Zalcman, N, Matzner, H & Priel, A 2016, 'Tyrosine Residue in TRPV1 Vanilloid Binding Pocket Regulates Deactivation Kinetics', *Journal of Biological Chemistry*, p. jbc. M116. 726372.

Lacolley, P, Regnault, V, Nicoletti, A, Li, Z & Michel, J-B 2012, 'The vascular smooth muscle cell in arterial pathology: a cell that can take on multiple roles', *Cardiovascular Research*, vol. 95, no. 2, pp. 194-204.

Laing, RJ & Dhaka, A 2015, 'ThermoTRPs and pain', *The Neuroscientist*, p. 1073858414567884.

Lam, DD, Przydzial, MJ, Ridley, SH, Yeo, GSH, Rochford, JJ, O'Rahilly, S & Heisler, LK 2008, 'Serotonin 5-HT2C Receptor Agonist Promotes Hypophagia via Downstream Activation of Melanocortin 4 Receptors', *Endocrinology*, vol. 149, no. 3, pp. 1323-8.

Larsen, PJ, Tang-Christensen, M & Jessop, DS 1997, 'Central Administration of Glucagon-Like Peptide-1 Activates Hypothalamic Neuroendocrine Neurons in the Rat*', *Endocrinology*, vol. 138, no. 10, pp. 4445-55.

Lee, TS 1954, 'Physiological gustatory sweating in a warm climate', *The Journal of Physiology*, vol. 124, no. 3, pp. 528-42.

Leung, FW 2008, 'Capsaicin-sensitive intestinal mucosal afferent mechanism and body fat distribution', *Life Sciences*, vol. 83, no. 1–2, pp. 1-5.

Li, B, Yin, Y, Liu, Y, Pi, Y, Guo, L, Cao, X, Gao, C, Zhang, L & Li, J 2014, 'TRPV1 activation impedes foam cell formation by inducing autophagy in oxLDL-treated vascular smooth muscle cells', *Cell Death & Disease*, vol. 5, no. 4, p. e1182.

Li, J & Wang, DH 2003, 'Function and regulation of the vanilloid receptor in rats fed a high salt diet', *Journal of Hypertension*, vol. 21, no. 8, pp. 1525-30.

Li, J & Wang, DH 2008, 'Increased GFR and renal excretory function by activation of TRPV1 in the isolated perfused kidney', *Pharmacological Research*, vol. 57, no. 3, pp. 239-46.

Liao, M, Cao, E, Julius, D & Cheng, Y 2013, 'Structure of the TRPV1 ion channel determined by electron cryo-microscopy', *Nature*, vol. 504, no. 7478, pp. 107-12.

Lin, L-Z, West, DP & Cordell, GA 1993, 'NMR Assignments of Cis- and Trans-Capsaicin', *Natural Product Letters*, vol. 3, no. 1, pp. 5-8.

Liu, Q, Zhang, J, Zerbinatti, C, Zhan, Y, Kolber, BJ, Herz, J, Muglia, LJ & Bu, G 2011, 'Lipoprotein Receptor LRP1 Regulates Leptin Signaling and Energy Homeostasis in the Adult Central Nervous System', *PLOS Biology*, vol. 9, no. 1, p. e1000575.

Ludy, M-J, Moore, GE & Mattes, RD 2011, 'The effects of capsaicin and capsiate on energy balance: critical review and meta-analyses of studies in humans', *Chemical Senses*.

Lukacs, V, Rives, J-M, Sun, X, Zakharian, E & Rohacs, T 2013a, 'Promiscuous Activation of Transient Receptor Potential Vanilloid 1 (TRPV1) Channels by Negatively Charged Intracellular Lipids THE KEY ROLE OF ENDOGENOUS PHOSPHOINOSITIDES IN MAINTAINING CHANNEL ACTIVITY', *Journal of Biological Chemistry*, vol. 288, no. 49, pp. 35003-13.

Lukacs, V, Yudin, Y, Hammond, GR, Sharma, E, Fukami, K & Rohacs, T 2013b, 'Distinctive changes in plasma membrane phosphoinositides underlie differential regulation of TRPV1 in nociceptive neurons', *The Journal of Neuroscience*, vol. 33, no. 28, pp. 11451-63.

Ma, L, Zhong, J, Zhao, Z, Luo, Z, Ma, S, Sun, J, He, H, Zhu, T, Liu, D & Zhu, Z 2011, 'Activation of TRPV1 reduces vascular lipid accumulation and attenuates atherosclerosis', *Cardiovascular Research*, vol. 92, no. 3, pp. 504-13.

Mary, G, Charles, C & Clifford, B 2006, 'Clinical laboratory parameters for Crl: CD (SD) rats', *Charles River Laboratories*, vol. 18.

Matsumoto, K, Kurosawa, E, Terui, H, Hosoya, T, Tashima, K, Murayama, T, Priestley, JV & Horie, S 2009, 'Localization of TRPV1 and contractile effect of capsaicin in mouse large intestine: high abundance and sensitivity in rectum and distal colon', *American Journal of Physiology - Gastrointestinal and Liver Physiology*, vol. 297, no. 2, pp. G348-G60.

Matsumoto, S, Nakanishi, R, Li, D, Alani, A, Rezaeian, P, Prabhu, S, Abraham, J, Fahmy, MA, Dailing, C, Flores, F, Hamal, S, Broersen, A, Kitslaar, PH & Budoff, MJ 2016, 'Aged Garlic Extract Reduces Low Attenuation Plaque in Coronary Arteries of Patients with Metabolic Syndrome in a Prospective Randomized Double-Blind Study', *The Journal of Nutrition*, vol. 146, no. 2, pp. 427S-32S.

McAllister, EJ, Dhurandhar, NV, Keith, SW, Aronne, LJ, Barger, J, Baskin, M, Benca, RM, Biggio, J, Boggiano, MM & Eisenmann, JC 2009, 'Ten putative contributors to the obesity epidemic', *Critical Reviews in Food Science and Nutrition*, vol. 49, no. 10, pp. 868-913.

McCarty, MF, DiNicolantonio, JJ & O'Keefe, JH 2015, 'Capsaicin may have important potential for promoting vascular and metabolic health', *Open Heart*, vol. 2, no. 1.

Mohapatra, DP & Nau, C 2005, 'Regulation of Ca2+-dependent desensitization in the vanilloid receptor TRPV1 by calcineurin and cAMP-dependent protein kinase', *Journal of Biological Chemistry*, vol. 280, no. 14, pp. 13424-32.

Morales-Lázaro, SL, Simon, SA & Rosenbaum, T 2013, 'The role of endogenous molecules in modulating pain through transient receptor potential vanilloid 1 (TRPV1)', *The Journal of Physiology*, vol. 591, no. 13, pp. 3109-21.

Moran, CP & Shanahan, F 2014, 'Gut microbiota and obesity: role in aetiology and potential therapeutic target', *Best Practice & Research Clinical Gastroenterology*, vol. 28, no. 4, pp. 585-97.

Morenilla-Palao, C, Planells-Cases, R, García-Sanz, N & Ferrer-Montiel, A 2004, 'Regulated exocytosis contributes to protein kinase C potentiation of vanilloid receptor activity', *Journal of Biological Chemistry*, vol. 279, no. 24, pp. 25665-72.

Mortensen, JM & Mortensen, JE 2009, 'The power of capsaicin', *Continuing Education Topics & Issues*, vol. 9, p. 8.

Movahed, P, Jönsson, BA, Birnir, B, Wingstrand, JA, Jørgensen, TD, Ermund, A, Sterner, O, Zygmunt, PM & Högestätt, ED 2005, 'Endogenous unsaturated C18 N-acylethanolamines are vanilloid receptor (TRPV1) agonists', *Journal of Biological Chemistry*, vol. 280, no. 46, pp. 38496-504.

Mózsik, G, Szolcsányi, J & Rácz, I 2005, 'Gastroprotection induced by capsaicin in healthy human subjects', *World Journal of Gastroenterology: WJG*, vol. 11, no. 33, p. 5180.

Mózsik, G, Past, T, Abdel Salam, OME, Kuzma, M & Perjési, P 2009a, 'Interdisciplinary review for correlation between the plant origin capsaicinoids, non-steroidal antiinflammatory drugs, gastrointestinal mucosal damage and prevention in animals and human beings', *Inflammopharmacology*, vol. 17, no. 3, pp. 113-50.

Mózsik, G, Dömötör, A, Past, T, Vas, V, Perjési, P, Kuzma, M, Blazics, G & Szolcsányi, J 2009b, *Capsaicinoids*, Budapest, Hungary: Akadémiai Publishing, pp. 18-22.

Muddhrry, PK, Ghatki, MA, Spokks, RA, Jonhs, PM, Pierson, AM, Hamid, QA, Kanse, S, Amara, SG, Burrik, JM, Legon, S, Polak, JM & Bloom, SR 1988, 'Differential expression of α-CGRP and β-CGRP by primary sensory neurons and enteric autonomic neurons of the rat', *Neuroscience*, vol. 25, no. 1, pp. 195-205.

Nakhate, KT, Kokare, DM, Singru, PS & Subhedar, NK 2011, 'Central regulation of feeding behavior during social isolation of rat: evidence for the role of endogenous CART system', *International Journal of Obesity*, vol. 35, no. 6, pp. 773-84.

Nazari, F, Ebrahimi, SN, Talebi, M, Rassouli, A & Bijanzadeh, HR 2007, 'Multivariate optimisation of microwave-assisted extraction of capsaicin from Capsicum frutescens L. and quantitative analysis by 1H-NMR', *Phytochemical Analysis*, vol. 18, no. 4, pp. 333-40.

Nelson, E 1919, 'THE CONSTITUTION OF CAPSAICIN, THE PUNGENT PRINCIPLE OF CAPSICUM', *Journal of the American Chemical Society*, vol. 41, no. 7, pp. 1115-21.

Niedzialkowska, E, Gasiorowska, O, Handing, KB, Majorek, KA, Porebski, PJ, Shabalin, IG, Zasadzinska, E, Cymborowski, M & Minor, W 2016, 'Protein purification and crystallization artifacts: The tale usually not told', *Protein Science*, vol. 25, no. 3, pp. 720-33.

Nikolopoulou, A & Kadoglou, NPE 2012, 'Obesity and metabolic syndrome as related to cardiovascular disease', *Expert Review of Cardiovascular Therapy*, vol. 10, no. 7, pp. 933-9.

Novelli, ELB, Souza, GA, Ebaid, GMX, Rocha, KKHR, Seiva, FRF, Mani, F, Campos, KE & Sforcin, JM 2010, 'Energy Expenditure and Oxygen Consumption as Novel Biomarkers of Obesity-induced Cardiac Disease in Rats', *Obesity*, vol. 18, no. 9, pp. 1754-61.

Nyman, M, Asp, NG, Cummings, J & Wiggins, H 1986, 'Fermentation of dietary fibre in the intestinal tract: comparison between man and rat', *The British Journal of Nutrition*, vol. 55, no. 3, pp. 487-96.

O'Neill, J, Brock, C, Olesen, A, Andresen, T, Nilsson, M & Dickenson, A 2012, 'Unravelling the mystery of capsaicin: a tool to understand and treat pain', *Pharmacological Reviews*, vol. 64, no. 4, p. 939.

Oliveira, FA, Vieira-Júnior, GM, Chaves, MH, Almeida, FR, Santos, KA, Martins, FS, Silva, RM, Santos, FA & Rao, VS 2004, 'Gastroprotective effect of the mixture of α-and β-amyrin from Protium heptaphyllum: role of capsaicin-sensitive primary afferent neurons', *Planta Medica*, vol. 70, no. 08, pp. 780-2.

Ollmann, MM, Wilson, BD, Yang, Y-K, Kerns, JA, Chen, Y, Gantz, I & Barsh, GS 1997, 'Antagonism of Central Melanocortin Receptors in Vitro and in Vivo by Agouti-Related Protein', *Science*, vol. 278, no. 5335, pp. 135-8.

Ono, K, Tsukamoto-Yasui, M, Hara-Kimura, Y, Inoue, N, Nogusa, Y, Okabe, Y, Nagashima, K & Kato, F 2011, 'Intragastric administration of capsiate, a transient receptor potential channel agonist, triggers thermogenic sympathetic responses', *Journal of Applied Physiology*, vol. 110, no. 3, pp. 789-98.

Orrenius, S, Gogvadze, V & Zhivotovsky, B 2015, 'Calcium and mitochondria in the regulation of cell death', *Biochemical and Biophysical Research Communications*, vol. 460, no. 1, pp. 72-81.

Pala, L, Monami, M, Ciani, S, Dicembrini, I, Pasqua, A, Pezzatini, A, Francesconi, P, Cresci, B, Mannucci, E & Rotella, CM 2011, 'Adipokines as possible new predictors of cardiovascular diseases: a case control study', *Journal of Nutrition and Metabolism*, vol. 2012.

Panahi, Y, Hosseini, MS, Khalili, N, Naimi, E, Simental-Mendía, LE, Majeed, M & Sahebkar, A 2016, 'Effects of curcumin on serum cytokine concentrations in subjects with metabolic syndrome: A post-hoc analysis of a randomized controlled trial', *Biomedicine & Pharmacotherapy*, vol. 82, pp. 578-82.

Panchal, SK & Brown, L 2011, 'Rodent Models for Metabolic Syndrome Research', *Journal of Biomedicine and Biotechnology*, vol. 2011, p. 351982.

Panchal, SK, Ward, L & Brown, L 2013, 'Ellagic acid attenuates high-carbohydrate, high-fat diet-induced metabolic syndrome in rats', *European Journal of Nutrition*, vol. 52, no. 2, pp. 559-68.

Panchal, SK, Poudyal, H, Waanders, J & Brown, L 2012, 'Coffee extract attenuates changes in cardiovascular and hepatic structure and function without decreasing obesity in high-carbohydrate, high-fat diet-fed male rats', *The Journal of Nutrition*, vol. 142, no. 4, pp. 690-7.

Panchal, SK, Poudyal, H, Iyer, A, Nazer, R, Alam, A, Diwan, V, Kauter, K, Sernia, C, Campbell, F, Ward, L, Gobe, G, Fenning, A & Brown, L 2011, 'High-carbohydrate High-fat Diet–induced Metabolic Syndrome and Cardiovascular Remodeling in Rats', *Journal of Cardiovascular Pharmacology*, vol. 57, no. 1, pp. 51-64.

Peña-Alvarez, A, Ramírez-Maya, E & Alvarado-Suárez, LÁ 2009, 'Analysis of capsaicin and dihydrocapsaicin in peppers and pepper sauces by solid phase microextraction–gas chromatography–mass spectrometry', *Journal of Chromatography A*, vol. 1216, no. 14, pp. 2843-7.

Por, Elaine D, Gomez, R, Akopian, Armen N & Jeske, Nathaniel A 2013, 'Phosphorylation regulates TRPV1 association with β-arrestin-2', *Biochemical Journal*, vol. 451, no. 1, pp. 101-9.

Poudyal, H, Panchal, S & Brown, L 2010a, 'Comparison of purple carrot juice and β-carotene in a high-carbohydrate, high-fat diet-fed rat model of the metabolic syndrome', *British Journal of Nutrition*, vol. 104, no. 9, pp. 1322-32.

Poudyal, H, Campbell, F & Brown, L 2010b, 'Olive Leaf Extract Attenuates Cardiac, Hepatic, and Metabolic Changes in High Carbohydrate–, High Fat–Fed Rats', *The Journal of Nutrition*, vol. 140, no. 5, pp. 946-53.

Prechtl, JC & Powley, TL 1990, 'The fiber composition of the abdominal vagus of the rat', *Anatomy and Embryology*, vol. 181, no. 2, pp. 101-15.

Reagan-Shaw, S, Nihal, M & Ahmad, N 2008, 'Dose translation from animal to human studies revisited', *The FASEB Journal*, vol. 22, no. 3, pp. 659-61.

Reddi, D, Curran, N & Stephens, R 2013, 'An introduction to pain pathways and mechanisms', *British Journal of Hospital Medicine (London, England: 2005)*, vol. 74, p. C188.

Reid, RC, March, DR, Dooley, MJ, Bergman, DA, Abbenante, G & Fairlie, DP 1996, 'A Novel Bicyclic Enzyme Inhibitor as a Consensus Peptidomimetic for the Receptor-Bound Conformations of 12 Peptidic Inhibitors of HIV-1 Protease', *Journal of the American Chemical Society*, vol. 118, no. 36, pp. 8511-7.

Robbins, N, Koch, SE & Rubinstein, J 2013, 'Targeting TRPV1 and TRPV2 for potential therapeutic interventions in cardiovascular disease', *Translational Research*, vol. 161, no. 6, pp. 469-76.

Rohacs, T 2015, 'Phosphoinositide regulation of TRPV1 revisited', *Pflügers Archiv - European Journal of Physiology*, vol. 467, no. 9, pp. 1851-69.

Rollyson, WD, Stover, CA, Brown, KC, Perry, HE, Stevenson, CD, McNees, CA, Ball, JG, Valentovic, MA & Dasgupta, P 2014, 'Bioavailability of capsaicin and its implications for drug delivery', *Journal of Controlled Release*, vol. 196, pp. 96-105.

Rong, W, Hillsley, K, Davis, JB, Hicks, G, Winchester, WJ & Grundy, D 2004, 'Jejunal afferent nerve sensitivity in wild-type and TRPV1 knockout mice', *The Journal of Physiology*, vol. 560, no. 3, pp. 867-81.

Russell, FA, King, R, Smillie, S-J, Kodji, X & Brain, SD 2014, 'Calcitonin Gene-Related Peptide: Physiology and Pathophysiology', *Physiological Reviews*, vol. 94, no. 4, pp. 1099-142.

Rüttimann, EB, Arnold, M, Hillebrand, JJ, Geary, N & Langhans, W 2009, 'Intrameal Hepatic Portal and Intraperitoneal Infusions of Glucagon-Like Peptide-1 Reduce Spontaneous Meal Size in the Rat via Different Mechanisms', *Endocrinology*, vol. 150, no. 3, pp. 1174-81.

Rzucidlo, EM, Martin, KA & Powell, RJ 2007, 'Regulation of vascular smooth muscle cell differentiation', *Journal of Vascular Surgery*, vol. 45, no. 6, pp. A25-A32.

Sadowska, J, Gębczyński, AK & Konarzewski, M 2017, 'Metabolic risk factors in mice divergently selected for BMR fed high fat and high carb diets', *PLoS ONE*, vol. 12, no. 2.

Salabei, JK & Hill, BG 2015, 'Autophagic regulation of smooth muscle cell biology', *Redox Biology*, vol. 4, pp. 97-103.

Saravanan, G, Ponmurugan, P, Deepa, MA & Senthilkumar, B 2014, 'Anti-obesity action of gingerol: effect on lipid profile, insulin, leptin, amylase and lipase in male obese rats induced by a high-fat diet', *Journal of the Science of Food and Agriculture*, vol. 94, no. 14, pp. 2972-7.

Savastano, DM, Hayes, MR & Covasa, M 2007, 'Serotonin-type 3 receptors mediate intestinal lipid-induced satiation and Fos-like immunoreactivity in the dorsal hindbrain', *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, vol. 292, no. 3, pp. R1063-R70.

Schwartz, MW, Woods, SC, Porte, D, Seeley, RJ & Baskin, DG 2000, 'Central nervous system control of food intake', *Nature*, vol. 404, no. 6778, pp. 661-71.

Sekar, S, Shafie, SR, Prasadam, I, Crawford, R, Panchal, SK, Brown, L & Xiao, Y 2017, 'Saturated fatty acids induce development of both metabolic syndrome and osteoarthritis in rats', *Scientific reports*, vol. 7, p. 46457.

Senning, EN, Collins, MD, Stratiievska, A, Ufret-Vincenty, CA & Gordon, SE 2014, 'Regulation of TRPV1 ion channel by phosphoinositide (4,5)-bisphosphate: the role of membrane asymmetry', *The Journal of Biological Chemistry*, vol. 289, no. 16, pp. 10999-1006.

Smutzer, G & Devassy, RK 2016, 'Integrating TRPV1 Receptor Function with Capsaicin Psychophysics', *Advances in Pharmacological Sciences*, vol. 2016, p. 16.

Someya, A, Horie, S, Yamamoto, H & Murayama, T 2003, 'Modifications of Capsaicin-Sensitive Neurons in Isolated Guinea Pig Ileum by [6]-Gingerol and Lafutidine', *Journal of Pharmacological Sciences*, vol. 92, no. 4, pp. 359-66.

Steinhoff, MS, von Mentzer, B, Geppetti, P, Pothoulakis, C & Bunnett, NW 2014, 'Tachykinins and Their Receptors: Contributions to Physiological Control and the Mechanisms of Disease', *Physiological Reviews*, vol. 94, no. 1, pp. 265-301.

Surh, Y-J & Sup Lee, S 1995, 'Capsaicin, a double-edged sword: Toxicity, metabolism, and chemopreventive potential', *Life Sciences*, vol. 56, no. 22, pp. 1845-55.

Suzuki, K, Jayasena, CN & Bloom, SR 2012, 'Obesity and appetite control', *Experimental Diabetes Research*, vol. 2012.

Szolcsányi, J 1977, 'A pharmacological approach to elucidation of the role of different nerve fibres and receptor endings in mediation of pain', *Journal de Physiologie*, vol. 73, no. 3, p. 251.

Szolcsányi, J 2015, 'Effect of capsaicin on thermoregulation: an update with new aspects', *Temperature*, vol. 2, no. 2, pp. 277-96.

Takenaka, T 2001, 'Classical vs reverse pharmacology in drug discovery', *BJU International*, vol. 88, pp. 7-10.

Tangsucharit, P, Takatori, S, Zamami, Y, Goda, M, Pakdeechote, P, Kawasaki, H & Takayama, F 2016, 'Muscarinic acetylcholine receptor M1 and M3 subtypes mediate acetylcholine-induced endothelium-independent vasodilatation in rat mesenteric arteries', *Journal of Pharmacological Sciences*, vol. 130, no. 1, pp. 24-32.

Tarling, EJ, de Aguiar Vallim, TQ & Edwards, PA 2013, 'Role of ABC transporters in lipid transport and human disease', *Trends in Endocrinology and Metabolism: TEM*, vol. 24, no. 7, pp. 342-50.

Thresh, I 1876, 'Capsaicin', *Analyst*, vol. 1, no. 8, pp. 149a-a.

Tolhurst, P, Lindberg, R, Calder, R & de Courten, M 2016, 'Australia's health tracker 2016.', *Melbourne: The Australian Health Policy*.

Tominaga, M, Caterina, MJ, Malmberg, AB, Rosen, TA, Gilbert, H, Skinner, K, Raumann, BE, Basbaum, AI & Julius, D 1998, 'The Cloned Capsaicin Receptor Integrates Multiple Pain-Producing Stimuli', *Neuron*, vol. 21, no. 3, pp. 531-43.

Torres-Narváez, JC, Mondragón, LdV, Varela López, E, Pérez-Torres, I, Díaz Juárez, JA, Suárez, J & Hernández, GP 2012, 'Role of the transient receptor potential vanilloid type 1 receptor and stretch-activated ion channels in nitric oxide release from endothelial cells of the aorta and heart in rats', *Experimental & Clinical Cardiology*, vol. 17, no. 3, pp. 89-94.

Vermeersch, S, Benschop, RJ, Van Hecken, A, Monteith, D, Wroblewski, VJ, Grayzel, D, de Hoon, J & Collins, EC 2015, 'Translational Pharmacodynamics of Calcitonin Gene-Related Peptide Monoclonal Antibody LY2951742 in a Capsaicin-Induced Dermal Blood Flow Model', *Journal of Pharmacology and Experimental Therapeutics*, vol. 354, no. 3, pp. 350-7.

Vriens, J, Appendino, G & Nilius, B 2009, 'Pharmacology of vanilloid transient receptor potential cation channels', *Molecular Pharmacology*, vol. 75, no. 6, pp. 1262-79.

Wagner, CE, Cahill, TM & Marshall, PA 2011, 'Extraction, Purification, and Spectroscopic Characterization of a Mixture of Capsaicinoids', *Journal of Chemical Education*, vol. 88, no. 11, pp. 1574-9.

Wagner, H & Bladt, S 1996, 'Drugs with Pungent-Tasting Principles', *Plant Drug Analysis: A Thin Layer Chromatography Atlas*, pp. 291-303.

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*, vol. 61, no. 8, pp. 2155-65.

Wang, Q, Ma, S, Li, D, Zhang, Y, Tang, B, Qiu, C, Yang, Y & Yang, D 2014, 'Dietary Capsaicin Ameliorates Pressure Overload–Induced Cardiac Hypertrophy and Fibrosis Through the Transient Receptor Potential Vanilloid Type 1', *American Journal of Hypertension*, vol. 27, no. 12, pp. 1521-9.

Wang, Y, Lomakin, A, Kanai, S, Alex, R, Belli, S, Donzelli, M & Benedek, GB 2016, 'The molecular basis for the prolonged blood circulation of lipidated incretin peptides: Peptide oligomerization or binding to serum albumin?', *Journal of Controlled Release*, vol. 241, pp. 25-33.

Wei, J, Ching, LC, Zhao, JF, Shyue, SK, Lee, HF, Kou, Y & Lee, TS 2013, 'Essential role of transient receptor potential vanilloid type 1 in evodiamine- mediated protection against atherosclerosis', *Acta Physiologica*, vol. 207, no. 2, pp. 299-307.

WHO 2000, 'General guidelines for methodologies on research and evaluation of traditional medicine', *World Health Organization*.

Woolf, CJ 2011, 'Central sensitization: implications for the diagnosis and treatment of pain', *Pain*, vol. 152, no. 3, pp. S2-S15.

Wu, LJ, Sweet, TB & Clapham, DE 2010, 'International Union of Basic and Clinical Pharmacology. LXXVI. Current progress in the mammalian TRP ion channel family', *Pharmacological Reviews*, vol. 62, no. 3, pp. 381-404.

Xu, X, Wang, P, Zhao, Z, Cao, T, He, H, Luo, Z, Zhong, J, Gao, F, Zhu, Z, Li, L, Yan, Z, Chen, J, Ni, Y, Liu, D & Zhu, Z 2011, 'Activation of Transient Receptor Potential Vanilloid 1 by Dietary Capsaicin Delays the Onset of Stroke in Stroke-Prone Spontaneously Hypertensive Rats', *Stroke*, vol. 42, no. 11, pp. 3245-51.

Yamato, E, Ikegami, H, Takekawa, K, Fujisawa, T, Nakagawa, Y, Hamada, Y, Uedo, H & Ogihara, T 1997, 'Tissue-Specific and Glucose-Dependent Expression of Receptor Genes for Glucagon and Glucagon-Like Peptide-1 (GLP-1)', *Hormones & Metabolic Research*, vol. 29, no. 02, pp. 56-9.

Yang, D, Luo, Z, Ma, S, Wong, WT, Ma, L, Zhong, J, He, H, Zhao, Z, Cao, T & Yan, Z 2010, 'Activation of TRPV1 by dietary capsaicin improves endothelium-dependent vasorelaxation and prevents hypertension', *Cell Metabolism*, vol. 12, no. 2, pp. 130-41.

Yang, H, Meijer, HG, Doll, RJ, Buitenweg, JR & Van Gils, SA 2016, 'Dependence of nociceptive detection thresholds on physiological parameters and capsaicin-induced neuroplasticity: a computational study', *Frontiers in Computational Neuroscience*, vol. 10.

Yao, J, Nair, M & Chandra, A 1994, 'Supercritical carbon dioxide extraction of Scotch Bonnet (Capsicum annuum) and quantification of capsaicin and dihydrocapsaicin', *Journal of Agricultural and Food Chemistry*.

Zahra, N, Alim-un-Nisa, IK, Hina, S, Javed, A, Inam, SM, Malik, SM & Arshad, F 2016, 'Estimation of capsaicin in different chilli varieties using different Extraction Techniques and HPLC method: A Review', *Pakistan Journal of Food Sciences*, vol. 26, no. 1, pp. 54-60.

Zhang, J & Ritter, RC 2012, 'Circulating GLP-1 and CCK-8 reduce food intake by capsaicin-insensitive, nonvagal mechanisms', *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, vol. 302, no. 2, pp. R264-R73.

Zhang, M-J, Yin, Y-W, Li, B-H, Liu, Y, Liao, S-Q, Gao, C-Y, Li, J-C & Zhang, L-L 2015, 'The role of TRPV1 in improving VSMC function and attenuating hypertension', *Progress in Biophysics and Molecular Biology*, vol. 117, no. 2, pp. 212-6.

Zhang, X, Zhuang, J, Wu, H, Chen, Z, Su, J, Chen, S & Chen, J 2010, 'Inhibitory Effects of Calcitonin Gene-Related Peptides on Experimental Vein Graft Disease', *The Annals of Thoracic Surgery*, vol. 90, no. 1, pp. 117-23.

Zhang, XN, Chen, DZ, Zheng, YH, Liang, JG, Yang, HX, Lin, XW & Zhuang, J 2009, 'Gene Transfer of Calcitonin Gene–Related Peptide Suppresses Development of Allograft Vasculopathy', *Transplantation Proceedings*, vol. 41, no. 5, pp. 1900-5.

Zheng, L-R, Zhang, Y-Y, Han, J, Sun, Z-W, Zhou, S-X, Zhao, W-T & Wang, L-H 2015, 'Nerve growth factor rescues diabetic mice heart after ischemia/reperfusion injury via up-regulation of the TRPV1 receptor', *Journal of Diabetes and its Complications*, vol. 29, no. 3, pp. 323-8.

Zvara, Á, Bencsik, P, Fodor, G, Csont, T, Hackler Jr, L, Dux, M, Fürst, S, Jancsó, G, Puskás, LG & Ferdinandy, P 2005, 'Capsaicin-sensitive sensory neurons regulate myocardial function and gene expression pattern of rat hearts: a DNA microarray study', *The FASEB Journal*, vol. 20, no. 1, pp. 160-2.