“Protein and Energy Metabolism in Cachexia and Obesity”

Submitted by

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A thesis submitted in total fulfillment of the requirements for the degree of Doctor of Philosophy

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March 2009

Acknowledgements

To my Mum, Dad, Aaron, John, Onni, Dante, William, Nonno and Zia Elizeberth for your love and care. Long have we talked, laughed and cried. Through my challenges you helped to bear, to listen in my trying times, I learnt the true value of our family and your priceless worth. To Joe for your staff of wisdom to guide the tough road, Chris for the understanding and caring words, Attila and Liam for a friendship and laughs. Ivan for the Judo spirit and vigor, Shaul for your guidance into the light. Paul, Xenia, all bretheren at 530 U.G.L.V. for support in their own forms. To my supervisors (Dr. Mark Jois, Associate Professor Richard Weisinger, Dr. John Power) for the opportunity to undertake and complete my thesis studies, your support and understanding over the long and enduring years. Lastly, in memories of Angelo Campeotto and Ben Kronenberg (OBM). Both taught me to live life to its fullest and pursue what dreams you are passionate about with every breath, without fear or regret.

For all those who thought I would amount to nothing…

"WHO said it could not be done? And what great victories has he to his credit which qualify him to judge others accurately?"
Napoleon Hill (b. October 25, 1883 – d. November 8, 1970 – American Author)

Patience, Persistence, Persipration …
"When you seek it, you cannot find it,
Your hand cannot reach it, nor your mind can exceed it.
When you no longer seek it; it is always with you"
Unknown Rōnin – Japan

The trying years in between commencing and finishing my thesis - Failure and Success …

"For everything you have missed, you have gained something else, and for everything you gain, you lose something else".
Ralph Waldo Emerson

"He who fears being conquered is sure of defeat".
Napoleon Bonaparte

Thesis Abbreviations

ABTS	2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ADP	Adenosine Diphosphate
Ad lib	Ad libitum
AIA	Acid Insoluble Ash
AIDS	Acquired Immune Deficiency
AMP	Adenosine Monophosphate
Ang II	Angiotensin II
APRP	Acute Phase Reactive Proteins
ATP	Adenosine Triphosphate
BAT	Brown Adipose Tissue
BCAA	Branched Chained Amino Acid
bpm	Beats Per Minute
BMA	Bone Mineral Area
BMC	Bone Mineral Content
BMI	Body Mass Index
BMR	Basal Metabolic Rate
cAMP	Cyclic Adenosine Monophosphate
cDNA	Complementary
CHF	Congestive Heart Failure
Da	Daltons
DEXA	Dual Energy X-ray Absorptiometry
DHA	Docosahexaenoic Acid
DIO	Diet Induced Obesity
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
EGCG	Epigallocatechin gallate
EPA	Eicosapentaenoic acid
EWAT	Epididymal Adipose Tissue
g	Grams
g	Gravitational force
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GH	Growth hormone
GLUT-4	Glucose Transport Protein 4
GoD	Glucose Oxidase
GTT	Glucose Tolerance Test
FA	Fatty Acids
FFA	Free Fatty Acids
H^+	Proton
HCl	Hydrochloric Acid
H_2SO_4	Sulphuric Acid
HSL	Hormone Sensitive Lipase
IGF-I	Insulin-like Growth Factor
IL-1	Interleukin 1
IL-6	Interleukin 2
i.v.	Intravenous
IMM	Inner Mitochondrial Membrane
kg	kilograms

L	Litre
LMF	Lipid Mobilizing Factor
LPL	Lipoprotein Lipase
LV	Left Ventricle
mL	Milliliter
µl	Microlitres
M	Molar
min	minute
MJ	Mega joule
mM	Millimolar
nCi	Nano Curies
NaOH	Sodium Hydroxide
NEFA	Non-esterified fatty acids
NPY	Neuropeptide Y
P	Probability
PF	Pair-fed
pg	picogram
PIF	Proteolysis Inducing Factor
POD	Horseradish Peroxidase
REE	Resting Energy Expenditure
RNA	Ribonucleic Acid
ROS	Reactive Oxygen Species
qRT-PCR	Quantitative Reverse Transcription Polymerase Chain Reaction
RVP	Right Ventricular Pacing
SAR-Ang II	Sarcosine Angiotensin II
SKM	Skeletal Muscle
SMR	Standard Metabolic Rate
S3	State III
S4	State IV
TNF-α	Tumor Necrosis Factor
UCP	Uncoupling Protein
WAT	White Adipose Tissue
/yr	per annum
<	Less than
>	Greater than
≡	Equivalent to
∑	The sum of

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Thesis Summary

The main objective of this thesis was to investigate the mechanistic role of uncoupling proteins (UCP's) to increase energy expenditure in two models of (i) Sarcosine- Angiotensin II (SAR-Ang II) infusion in rats and (ii) mechanical induced right ventricle pacing (RVP) in sheep.

Initially, a novel animal model concerning the effect of RVP on body composition, weight and composition changes and whole body palmitate turnover were investigated in adult sheep over an 8-week period. Next, a model of Angiotensin II (Ang II) infusion was used to induce weight loss and increased energy expenditure in adult female Sprague Dawley rats. Recovery from infusion was also observed. Changes in gene expression of uncoupling protein 3 (UCP3) in the skeletal muscle were studied using quantitative reverse transcription polymerase chain reaction (qRT-PCR) analysis. Both models displayed evidence of weight loss and increased energy expenditure. However this increased energy expenditure was shown not to be due to UCP 3 mRNA expression.

In addition, the influences of various compounds on long-term, diet induced obesity were examined with a view to compare mechanisms involved in both cachexia and obesity. Addition of green tea to a high fat diet, attentuated weight and adipose tissue gain, increased plasma non-esterified fatty acids (NEFA) and skeletal muscle gain. This result demonstrates a promotion of lean tissue body composition and weight loss. These findings suggest that nutritional supplements can be added to the diet to protect against Diet Induced Obesity (DIO) development by altering fat metabolism by decreasing lipogenesis, and increasing lipolytic pathways.

In conclusion, these studies identify that UCP 3 expression in skeletal muscle may not be the sole key mechanism explaining elevated energy expenditure during cardiac cachexia. Furthermore, green tea was used as a dietary supplement to promote weight loss, fat loss, lean tissue growth. Thus, this finding highlights the need to develop a set of hypotheses to explain the metabolic mechanisms behind this weight loss. These mechanisms may regulate both cardiac cachexia and development of diet induced obesity.

Statement of Authorship

"Except where reference is made in the text of the thesis, this thesis contains no material published elsewhere or extracted in whole or in part from a thesis submitted for the award of any other degree or diploma.

No other person's work has been used without due acknowledgement in the main text of the thesis.

This thesis has not been submitted for the award of any degree or diploma in any other tertiary institution.

That all research procedures reported in this thesis were approved by the relevant Ethics or Safety Committee or authorized officer as appropriate."

1.0 Introduction

The purpose of this literature review is to acquaint the reader with the condition known as cachexia, animal models of cachexia, and to compare cachexia with associated conditions such as starvation and sarcopenia. In this review, there will be discussion of cachexia in a very broad context. There will be mention of different types of cachexia, diagnosis and prognosis. Further, there are a number of mediators discussed, both anabolic and catabolic, which regulate body weight. Their role in cachexia will also be reviewed. Moreover, the role of Angiotensin II (Ang II) will be discussed in relation to congestive heart failure (CHF) and cardiac cachexia. On the other end of the weight spectrum, obesity appears to be related. In fact, cachexia and obesity are the end-points of physical manifestation of the body composition and weight spectrum. The use of antioxidants found in green tea, cocoa and coffee to alleviate diet-induced obesity will be explored. The literature provided, supports an understanding of the research presented in later chapters. The literature presented will provide solid groundwork for the studies undertaken in this thesis, so the reader may appreciate the experimental design, results and discussion of the key findings.

Repeating earlier statements, cachexia and obesity are physical expressions of an imbalance in the anabolic and catabolic factors which regulate body weight and composition. Both conditions display alterations in cytokine hormones, (e.g., tumour necrosis factor (TNF-α), neuropeptide Y (NPY), and growth hormone (GH)). In addition, the effects of Ang II on wasting will be reviewed and also various related conditions which cause weight loss (i.e., cancer, sarcopenia and starvation) will also be introduced. Overall, there is an urgent need for development of animal models of CHF cachexia, in

particular the elucidation of the mechanisms causing elevated energy expenditure during this condition. Lastly, the role of antioxidants in preventing obesity will be discussed. These antioxidants are found in a number of food products (i.e., cocoa, coffee, caffeine and green tea); with particular mention to green tea catechins, which modulate body composition, and thus may elevate diet induced obesity when included as a dietary supplement.

This review will not set out to explain all facets of weight loss, cachexia, associated diseases, obesity or antioxidants. The review focuses on the relationship between obesity and cachexia, malnutrition and anorexia, cachexia prognosis, sarcopenia (wasting during aging), key mediators of cachexia, different forms of cachexia, the role of Ang II in cardiac cachexia, diet induced obesity and role of anti-oxidants in obesity treatment. There is also some discussion of practical tools used in cachexia treatment.

Elevated energy expenditure is one of the main characteristics of cachexia and it is also suppressed in the obese state. The role of UCP's are reviewed as they are previously found to be altered in both the cachectic and obese states and effect energy expenditure.

1.1 Cachexia

Cachexia (Greek 'Kakos' and 'Hexis'; lit. 'Bad Condition') is a debilitating condition which is characterized by severe muscle emaciation and also adipose tissue loss and not explained solely by reduced food intake. Various studies have shown cachexia to be a metabolic disorder that is typified by elevated Resting Energy Expenditure (REE), increased catabolic hormone profile (i.e., catecholamines) and cachectic factors which often lead to premature death (Argiles, et al., 1997a; Frayn, 1991; Lorite, et al., 1998; Meadows, et al., 2000). It is further characterized by dysfunction of physiological processes in carbohydrate, fat and protein metabolism. Typically, cachexia is prevalent as the end point of many chronic diseases (i.e., CHF, cancer, auto-immune-deficiency syndrome (AIDS), arthritis, chronic renal failure, cystic fibrosis, chronic gastrointestinal disease, Crohn's disease, rheumatoid arthritis, sarcopenia, tuberculosis and sepsis) and occasionally surgery and injury (Argiles, et al., 2003; Belizario, et al., 1991; Frayn, 1991; Homma, et al., 1993; Tisdale, 2000).

Wasting due to adenocarcinoma is almost entirely due to increased sympathetic activation of thermogenesis (Frayn, 1991). In addition, during rheumatoid arthritis, the metabolic activity of loss of body cell mass, contributes up to 95% of the body's metabolic activity (Roubenoff, 2004). Thus, elevation of energy expenditure is a key indicator in the prognosis of the disease. A positive facet of this hypothesis relates that during infection, cachexia may represent a useful, primitive reflex to mobilize endogenous fuel and inhibit infection via thermogenesis (Frayn, 1991).

Cachexia is broadly accepted as the severe wasting associated with disease states such as cancer or AIDS, but a universally accepted and more detailed definition is

needed. Cachexia can be differentiated from starvation as during starvation there is a loss of body fat and non-fat mass due to inadequate protein and energy intake (Thomas, 2007). Efforts must be made to investigate as accurately as possible the diagnosis and prognosis of cachexia using existing information or developing novel animal models.

1.2 Cardiac Cachexia

Cardiac cachexia is the cachexia associated with cardiac failure. The aetiology of CHF is characterised by a fundamental dysfunction of the heart (e.g., enlargement of the left ventricle, severe systolic and diastolic dysfunction), and rapid or irregular pacing (e.g., tachycardia or right ventricular pacing) which may be caused by increased blood pressure. CHF is associated with anaemia, increased mortality and morbidity, a higher β-natriuretic peptide level, increased extracellular volume and deterioration of renal function. Anaemia worsens left ventricular hypertrophy, myocardial infarction and chronic coronary heart disease (Silverberg, et al., 2006).

Cardiac cachexia is defined as the non-oedematous weight loss in CHF (Anker & Coats 1999), with patients also experiencing atrial fibrillation, loss of appetite and fullness, anorexia, liver function abnormalities and altered fat absorption (King, et al., 1996a), with multifactorial aetiology (King, et al., 1996b). Cardiac cachexia can be divided into two types; e.g., (1) the classic type occurring in patients with severe heart failure; and (2) the post-operative type. In addition, cardiac cachexia may partially be due to decreased nutrient intake (e.g., anorexia, malabsorption) and specific metabolic alterations (e.g., hyper catabolism, response to hypoxia, inflammatory status - cytokines) (Mustafa & Leverve, 2001).

The process of heart failure appears to be a common and coordinated response to cardiac injury and dysfunction. Cardiac remodelling is the restructuring and reshaping of the heart that underlies heart failure progression, and is the major determinant of the clinical course of CHF, irrespective of its aetiology. Notably, the accumulating data in regard to both animal and human hearts suggesting cardio myocyte regeneration and renewal, indicate that cellular remodelling is a complex and dynamic process that is not completely understood (Fedak, et al., 2005).

The incidence of CHF is increasing in Westernized countries, marked by malnutrition, body weight loss of >7.5% in 6 months may be ascribed to neurohormonal alterations, increased energy requirements, and decreased activity, decreased muscle mass and strength due to atrophy of type 2a and 2b fibres (Bourdel-Marchasson & Emeriau, 2001).

In a review paper by (von Haehling, et al., 2007), researchers propose a number of mechanisms for wasting in cardiac cachexia, which include; Increased energy expenditure, neurohormonal activation and immune activation; and alteration of nutritional intake via decreased feed intake and bowel dysfunction.

These effects triggers further wasting and the progression of the wasting is further potentiated by micro and macro-nutrient deficiencies (von Haehling, et al., 2007). Wasting has also been proposed to be attributed to cellular hypoxia. Cellular hypoxia is a consequence of hormonal and cytokine activation (von Haehling, et al., 2007). The researchers further discuss the origin of increased energy expenditure being due to increased cardiac, ventilatory work and increased peripheral oxygen consumption.

Agreeing with the previous hypothesis, Anker & Coats (1999) proposes that patients with CHF have metabolic abnormalities, which lead to a catabolic syndrome and progressive loss of skeletal muscle in advanced stages of the disease. This skeletal muscle loss is correlated to neurohormonal and immunological profile (Anker, et al., 1997), whilst individuals have an increased REE for their metabolic size contributing to weight loss (Poehlman, et al., 1995).

Hormonal balance is a key factor modulating CHF cachexia. Cardiac cachexia is marked by reduced expression of IGF-I in the skeletal muscle (Schulze & Spate, 2005), elevated epinephrine, norepinephrine, ketone bodies, lactate, cortisol, renin activity, aldosterone plasma concentrations, lowered sodium levels, elevated FFA, TNF-α and decreased growth hormone (Anker, et al., 1997; Anker & Coats, 1999; Lommi, et al., 1998). In addition, patients have elevated glucagon, and adiponectin, and decreased ghrelin (Norrelund, et al., 2006). This modulation in hormonal profile leads to alterations in energy homeostasis and fat metabolism. REE, FFA (Free Fatty Acid) turnover and FFA oxidation are elevated in CHF cachexia, and are inversely correlated to left ventricular (LV) ejection fraction (Lommi, et al., 1998).

The falling cardiac output activates compensatory mechanisms (e.g., elevated catecholamines; Ang II), which assists to increase cardiac output, however high Ang II concentrations cause cardiac-myocyte necrosis, mononuclear cell activation, and unbalanced cytokine profile (Mann & Young, 1994). Further, patients experiencing long-term, neuro-hormonal/ autocrine-paracrine activation also display enlargement of left ventricle (LV) with lowered ejection fraction, chamber geometric alterations, deteriorated

pump performance, abnormal myocyte growth and elevated energy metabolism (Eichhorn & Bristow, 1996; King, et al., 1996a).

When cardiac wasting exceeds dietary energy and protein intake, there is consumption of existing adipose and skeletal muscle tissue (Meadows, et al., 2000) , with weight loss not being able to be alleviated solely by increased parenteral protein supplementation (Frayn, 1991). Emerging data indicate that conventional cardiovascular risk factors (e.g., hypercholesterolemia and obesity) are associated with better survival in patients with wasting. This may be the result of hemo-dynamic stability, protective adipokine profile, toxin sequestration of adipose tissue, and antioxidation capacity of muscle in obesity (Galinier, et al., 2005; Kalantar-Zadeh, et al., 2007).

Another contributing factor to CHF wasting is bowel dysfunction. CHF is a multi-organ disease with increasing evidence for the involvement of the gastrointestinal system (Krack, et al., 2005). Malnutrition may influence CHF directly, increasing mortality and morbidity, especially in elderly patients; affecting cardiac morphology and function (King, et al., 1996a). CHF may be treated using Angiotensin Converting Enzyme inhibitors and β-blockers (i.e., partially reversing systolic dysfunction) which retards the progression of LV dysfunction (Eichhorn & Bristow, 1996).

1.3 Cancer anorexia/ Cachexia syndrome

Epidemiological studies show that cancer cachexia is seen in two thirds of cancer patients and accounts for approximately one quarter of all cancer deaths (Argiles, et al., 1997a). Other estimates suggest cancer cachexia affects up to 50% of cancer patients during treatment (Norton, et al., 1987), thus adding to the increased morbidity and mortality of cancer patients. Further animal and cell studies have shown that cancer cachexia is associated with alterations in carbohydrate, lipid and protein metabolism. These alterations may be caused by tumour catabolic effects and altered tumour glucose requirements and ultimately, the host-mediated response to the tumour (Hirai, et al., 1997; Tisdale, 2000)

Cancer cachexia is a chronic syndrome with pronounced multi-faceted aetiology. Cancer cachexia is marked by elevated cytokine hormones, appetite satiety and increased REE (Collins, et al., 2002). Further, the patient displays rapid depletion of body adipose tissue reserves during disease progression (i.e., lung cancer) (Russell, et al., 2002), and elevated hepatic APRP (i.e., C-reactive protein) (Tisdale, 2004). Typically, the syndrome is characterised by marked weight loss, physical and mental fatigue, immuno-suppression, increased REE, anorexia, malnutrition, asthenia and anaemia, and accelerated catabolism state. The degree of cachexia is inter-related to the tumour growth, with the cachexia severity correlated to patient survival time (Argiles, et al., 1997a; Argiles, et al., 2003; Rubin, 2003) and inhibition of protein synthesis (Tisdale, 2002). Further, patients suffer increased protein degradation (Bossola, et al., 2001) due to elevation of pro-inflammatory cytokines (e.g., TNF-α, IFNγ, IL-6, PIF and ghrelin) (Garcia, et al., 2005). Thus, these studies suggest the tumour may initiate cachexia

through tumour endogenous proteins/ factors or the host inflammatory response to the tumour.

Cancer cachexia is a particularly common variant of cachexia, most prominent in patients with pancreatic or stomach carcinomas (Cariuk, et al., 1997). The mechanism of proteolysis in cancer cachexia has been suggested to be via specific protein degradation (via ATP-ubiquitin- dependent proteolytic pathway), correlated with the presence of catabolic factors (i.e., PIF) (Rubin, 2003; Tisdale, 2000). Proteolysis and associated hypermetabolism may be influenced by proteins of hepatic origin. Hepatic acute phase proteins have been considered to be markers of hyper-metabolism in cancer patients (Wigmore, et al., 2000). Hypermetabolism is a direct cause of irreversible weight loss. In cancer, two-thirds of patients experience weight loss due to tumour burden (i.e., the tumour produced factors impair protein synthesis) and shorter survival time (Tisdale, 2002). Unfortunately, nutrient supplementation and other appetite-manipulating drugs are unable to restore body weight (Argiles, et al., 1997a; Tisdale, 2004). Increased protein intake causes increased adipose tissue deposits in tumour bearing and non-tumour bearing rats (McCarthy, et al., 1997), thus altering body composition (e.g., development of obesity).

Anorexia experienced during cancer on the other hand is an effect rather than a cause of the cachexia; with skeletal muscle and adipose tissue catabolism supplying the body's energy requirements (Rubin, 2003; Tisdale, 2004). Body composition changes during cachexia resemble those changes found in infection and injury rather than starvation (Tisdale, 2004). Unlike other forms of cachexia, cancer cachexia utilizes a greater extent of carcass lipid. This lipid catabolism is caused by a tumour origin lipid

regulatory factors (Todorov, et al., 1998); however there is still some degree of lean body mass wasting (Nixon, et al., 1980). Specifically, cardiac protein is affected which also affects cardiac performance (Argiles, et al., 1997a).

1.4 Obesity and Cachexia – non-identical twins

In general terms, obesity and cachexia appear to be non-identical, and are both associated with adverse disease outcomes relating to body weight. However, a new paradigm has emerged which increases the scope to inter-relate the two conditions. This interrelationship is based on hormonal and genetic markers, and the degree of mortality.

Studies of patients who suffer from CHF revealed that those patients who were overweight or obese actually had improved survival outcomes compared to their leaner counter-parts (Curtis, et al., 2005; Davos, et al., 2003). As cachexia is an underlying condition of a wide variety of disease states (e.g., cancer, heart failure, AIDS), it is best defined by its hallmark features. These include; anorexia, fatigue, increased body temperature, and increased REE, skeletal muscle wasting, increased acute-phase reactive protein (APRP) production and weight loss (Kotler, 2000), although (Anker & Coats, 1999) found it quite difficult to draw together as a clear definition. They concluded that there is an array of indicators used to determine weight loss and body composition (e.g., cachexia was apparent when body fat was <29% for women and <27% for men), but generally diagnosis differs in complexity and definition depending on the authour. In comparison, the general definition for obesity has shifted with time, but at present may be assumed to be defined as excessive of body fat (Dehghan, et al., 2005). A Pubmed search (December 2008) of the term ‘cachexia and obesity’ yielded 202 articles in publication.

However, as single terms they yielded 4833, and 115146 articles respectively. Thus, there is a need to research the links between the two disease states more closely.

The general hypothesis proposed to link the two diseases states that during obesity and to some degree cachexia, there is increased adiposity, associated with insulin resistance, particularly the visceral component of the body, resulting in increased free-fatty acid flux (Heilbronn, et al., 2004). Further, in moderate obesity there is some evidence of proteolysis (Jensen & Haymond, 1991), as also seen in cancer cachexia (Tisdale, 2003). In addition, the adipocyte is known to secrete a number of hormones, which act directly or indirectly on the myocardium (i.e., Ang II, leptin, resistin, adrenomedulin, cytokines) (Galinier, et al., 2005), possibly also being involved in cachexia prognosis (see Fig. 1.1).

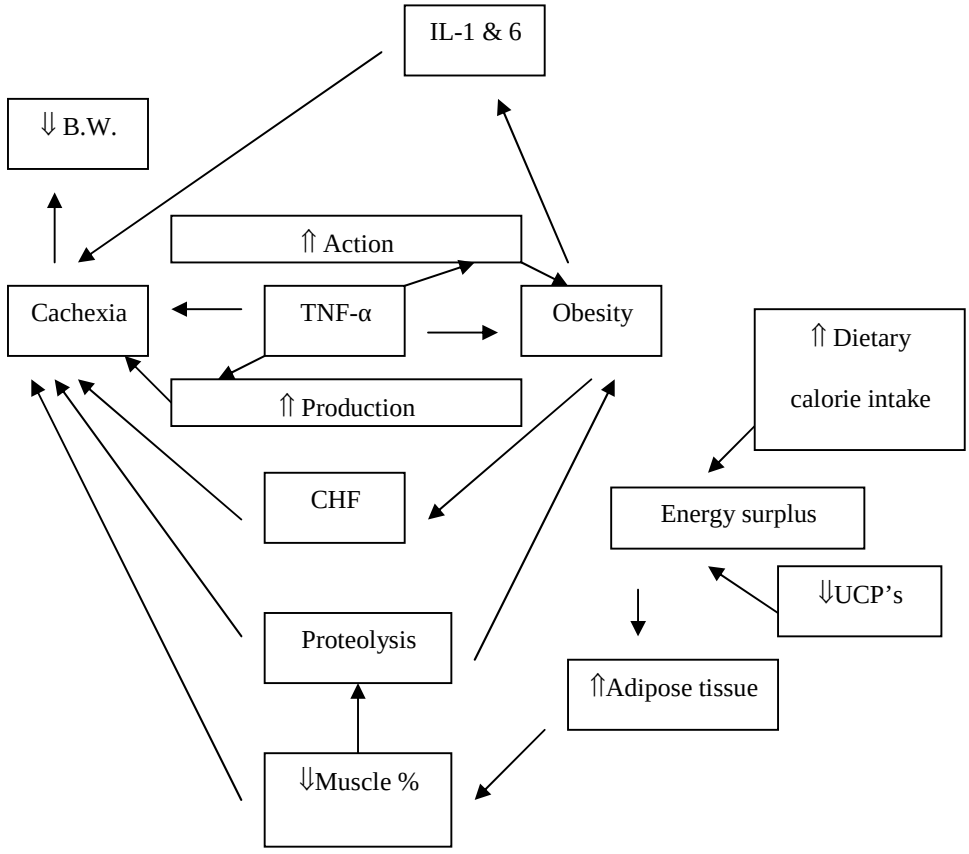
Cytokine hormones have a central role in both cachexia (e.g., cytokine release under hypothalamic control, and immune cells activation) and in obesity (e.g., interleukin 1 (IL-1) and interleukin 6 (IL-6) are released) (Guijarro, et al., 2006). TNF-α has been proposed to be pivitol cytokine playing a key role in both cachexia and obesity, decreasing body mass via its increased production and action (during cachexia) and action (during obesity) (Argiles, et al., 1997b) (see Fig. 1.1)

Over the past few years, this inter-connecting hypothesis has formed the basis of research exploring the link between the two conditions. A multitude of studies has explored this hypothesis and has produced a growing number of research ideas, which includes both human and animal models used to study the conditions.

Figure 1.1 The inter-relationship connecting cachexia and obesity

Explanatory Legend

IL = Interleukin, **TNF=** Tumour Necrosis Factor, **UCP =** Uncoupling Proteins, **B.W. =** Body Weight, ⇓ = decreases, ⇑ = increases **Arrows** (⟶) depict what occurs during the specific effect or disease state (e.g., there is increased production & action of TNF-a in cachexia which also causes decreased B.W.)



1.5 Anorexia and Cachexia – Concepts in malnutrition

Human epidemiological studies and animal experimentation have shown that anorexia and cachexia are independent conditions that may share similarities. During adulthood in humans, deviation of greater than 10% body weight is related to increased mortality (Anker and Coats 1999). Both cachexia and starvation may lead to anorexia and are two facets of malnutrition (Kotler, 2000). Anorexia is typically defined as caloric restriction (starvation) leading to weight loss; as the individual retains lean mass at the expense of increased fat metabolism, with weight gain possible upon re-feeding (Kotler, 2000; Todorov, et al., 1999). Further, anorexia is due to excessive mobilization of oxidisable fuel from adipose and other tissues, which also influences food intake (Friedman, 1998). In contrast, cachexia is associated with elevated inflammation response or tumour conditions where re-feeding or parentaral nutrient cannot reverse the weight loss (Kotler, 2000). Moreover, nutrient intake (e.g., imbalanced amino acid intake) may also cause anorexia and weight loss (Edelman, et al., 1999), but not cachexia. In addition, anorexia mainly leads to loss of fat tissue and reduced plasma albumin levels, yet cardiac cachexia patients undergo loss of fat, muscle and bone, without alteration in albumin and liver enzymes (Anker and Coats 1999).

Interestingly, anorexia may also overlap with cachexia (i.e., during cancer cachexia), which may cause hypo-phagia, depressed serum insulin-like growth factor (IGF-I) and depressed appetite (McCarthy, et al., 1997). Anorexia also occurs as a contributing factor during cachexia, and its presence is mainly attributed to reduced appetite or inability to eat, mainly seen in cachectic cancer (gastrointenstinal) patients

(Tisdale, 1997). Tisdale (1997) further proposes satietins as possible candidates for the occurrence of the two conditions concurrently, causing dysfunction of the hypothalamic NPY feeding system. Interestingly, only in cachexia with aging (i.e., sarcopenia), is there a display of subnormal levels of skeletal muscle without body weight loss (Kotler, 2000), which is not present in anorexia.

1.6 Sarcopenia (Wasting with Aging) and energy expenditure

Wasting also occurs with age, and is termed "sarcopenia" and epidemiological studies have shown that sarcopenia commences at age ~25, with lean body mass declining at 0.3kg/yr (Kuczmarski, 1989)**.** *Sarcopenia* (*Gk* 'poverty of flesh'), is defined as the loss of muscle mass and strength with age, causing frailty and disability and is potentiated by illness (Roubenoff, 2004). In comparison to cachexia, sarcopenia is a more chronic form of wasting spanning many years. Obesity in the elderly acts synergistically with sarcopenia to enhance disability (i.e., "fat frail") – increased weakness and weight combination (e.g., decreasing muscle and increasing fat mass). Sarcopenia is responsible for diminished physical activity, positive energy balance, obesity as muscle mass decreases (i.e., reduced insulin sensitive tissues), TNF-α and IL-6 all leading to elevated insulin resistance and proteolysis (Roubenoff, 2004). Sarcopenia is considered a separate condition from anorexia and cachexia as the individual has subnormal levels of skeletal muscle tissue as they age, with rare occurances in patients experiencing growth hormone deficiency (Kotler, 2000). Sarcopenia occurs synergistically with increased adipose tissue stores (Anker & Coats 1999). Animal studies have subsequently shown that age-related hypertrophy of adipose tissue has been associated with a significant decrease in the

number of Ang II AT(1) receptors and in obese rats, and with decreased receptor binding (Pinterova, et al., 2001). These findings have important implications for the community population health as sarcopenia may augment the obesity epidemic, thus becoming an increasingly common, non-communicable disease which may underlie increased disability and mortality in an aging population.

Obesity and sarcopenia are similar in that the two related conditions both arise from dysfunction of energy, protein and fat metabolism. They are great epidemiological trends of our times, altering body composition (e.g., increased fat mass) and mortality (e.g., shorter life-span). ROS formation is particularly high in the cellular energy-generating particles; mitochondria, where oxygen serves to generate essential biochemical reactants (i.e., ATP), and require antioxidants (Weisburger, 2001). A number of systems that generate oxygen free radicals catalyse the oxidative modification of proteins. Protein oxidation contributes to the pool of damaged enzymes, which increases in size during aging and in various pathological states. Protein oxidation rate is also affected by the age-dependent accumulation of unrepaired DNA damage (Stadtman, 1992). Skeletal muscle generates oxygen species at a number of sub-cellular sites and oxygen species increase with contractile activity (Jackson, 2008). Muscle mass and maximal force are decreased with increasing ROS production in elderly rats (Chabi, et al., 2008), leading to reduced myocyte motor units, progressive denervation, atrophy, and significant reduction in regenerative potential (Fulle, et al., 2005; Fulle, et al., 2004). Thus, ROS production may influence the development of both sarcopenia and obesity.

Despite the number of studies conducted to examine the cause and treatment of sarcopenia, the origin of hypermetabolism and the physiological processes governing it need to be determined and have yet to be fully understood.

1.7 Diagnosis of wasting

Human epidemiological studies have now clearly shown that the guideline for the diagnosis of cachexia seems complex and unclear. The research conducted mostly focuses on both weight loss and time period of the weight loss as key indicators of cachexia; however, identification of the specific physiological mechanisms which underlie this relationship, necessary (e.g., energy expenditure, weight loss correlation to body composition, cytokine hormones and gene expression markers). The diagnosis of malnutrition seems problematic as the nutritional intake i.e., may vary with disease progression (reduced food intake) and thus body weight loss is seen as a key measure to predict cachexia (Kotler, 2000). Body weight loss of greater than 10% over a 1-year period is considered abnormal (Kotler, 2000). In contrast, the use of body composition measures entails a more subtle analysis of cachexia. The loss of >7.5% of non-intentional and non-oedematous weight over 6-months of pre-morbid weight in cardiac cachexia is considered ‘abnormal’ (Anker and Coats 1999). During cachexia, there is a wide range of immune abnormalities present (e.g. increased plasma TNF-α, combined with IL-1, IL-6, interferon-γ and transforming growth factor-β), (see Fig. 1.1) which are believed to indicate advanced catabolism and also to suggest the probability of survival of a patient with cachexia (Anker and Coats 1999). While providing an indicative measure of cachexia, weight loss is governed by an imbalance between anabolic and catabolic pathways (e.g., decreased ghrelin, GH, muscle insulin-like growth factor (IGF-I) and

increased leptin) (von Haehling, et al., 2007). There are a number of mechanisms which underlie the catabolic pathways in different forms of cachexia. Mice with implanted MAC16 tumour display increased expression of 20S proteasome α-subunits, the p42 regulator, and ubiquitin-conjugating enzyme (E214k) (Whitehouse & Tisdale, 2003). Moreover, a study of mature subcutaneous fat cells and differentiated pre-adipocytes in cancer patients with cachexia revealed that there was a 40% reduction in body fat and a 2-fold increase *in vivo* lipolysis rate. The lipolytic effects of catecholamine and natriuretic peptide were increased by 2-3-fold in cachexia and completely suppressed by the inhibition of HSL, which is increased by 100% in its protein activity during cachexia (Agustsson, et al., 2007). In cancer cachexia, there generally is wasting of both body muscle and fat. On the other hand, in CHF cachexia, there is more wasting of skeletal muscle and increased visceral adipose tissue. Thus, diagnosis of cachexia is multifactorial and involves patient history information regarding cytokine hormones and weight loss parameters. The analysis of the weight loss into individual body composition components may shed more light on diagnosis of cachexia.

1.8 Body composition re-modelling during Cachexia

The remodelling body composition that occurs during cachexia involves direct physical changes in the number of tissue cells (e.g., skeletal muscle, fat and bone) and cell type, which influences modification in organ system size of metabolically active tissue (e.g., liver, heart) and the function of those cells. During cachexia the power output of skeletal muscle as a function of muscle mass and strength is diminished as muscle fatigue commences (Tisdale, 2003). The body composition re-modelling is influenced by the increased presence of cytokine hormones and changes in fat synthesis and degradation of proteins. The effects of these anabolic and catabolic processes have been discussed by (Kotler, 2000), who notes that both fat versus fat-free loss are observed during cachexia, but the loss is mostly from extra-cellular water volume, and that decreases in intracellular potassium concentrations indicates a bio-energetic deficiency. In a study using pair-fed animals, it has been observed that skeletal mass weight loss could not be explained by decreased muscle mass alone (Kotler, 2000). Many examples of body composition remodelling have been identified in various cachexia disease states in human and animal studies. There are a wide variety of examples where these changes take place, including Crohn's disease in children (Burnham, et al., 2005)**,** cardiac cachexia (Anker and Coats, 1999) and many more conditions.

In cachexia, and in particular cardiac cachexia, patients lose not only muscle mass but they also experience loss of fat and bone mineral density (von Haehling, et al., 2007), however regional fat (e.g., visceral may) increase (*Pers. Comm* M.Jois 2004). Similarly, another contributing factor to decreased body weight is dehydration due to poor fluid intake (Morley, et al., 2006). Cachexia is characterized by selective skeletal muscle

depletion, with exact mechanisms of the depletion still to be precisely elucidated. Another example of body composition re-modelling occurs in mice bearing the MAC16 tumour. The mice were given a supplement of a polyunsaturated fatty acid; eicosapentaenoic acid (EPA), which attenuated body weight loss and suppressed protein catabolism in *soleus* muscles (Whitehouse & Tisdale, 2003). The inhibition of protein metabolism was achieved through inhibition of an ATP-dependent proteolytic pathway and expression of 20S proteasome a-subunits and the p42 regulator (Whitehouse & Tisdale, 2003). In another mouse model, (Samuels, et al., 2001) demonstrated that mice with cancer cachexia (induced by colon 26, adenocarcinoma), mice experienced skeletal muscle loss, which was mediated by both decreased (238%) protein synthesis and increased (1131%) degradation. However, in a later experiment, cachexia was solely caused by decreased protein synthesis (~260%) in mice that underwent chemotherapy (cystemustine) treatment (Samuels, et al., 2001).

The loss of fat mass that appears to be a key feature of cancer cachexia is mainly attributed to increased lipolysis in adipocytes. Significant alterations in fat metabolism occurred in 26 cancer patients with and without cachexia. The patients displayed display increased levels of catecholamines and natriuretic peptides and as a result the patients with cachexia experienced a two-fold increase in lipolysis (e.g. via HSL) (Agustsson, et al., 2007). HSL is an intracellular neutral lipase, capable of hydrolyzing a variety of esters (e.g., 1st fatty acid from a triacylglycerol molecule), and freeing a fatty acid and diglyceride. Only the initial enzyme step is affected by hormones giving rise to its name (Kraemer & Shen 2002). The inhibition of hormone-sensitive lipase (HSL) inhibited

lipolysis in adipocyte in cancer cachexia is proposed as a potential target to prevent weight loss (Agustsson, et al., 2007).

In summary, the re-organisation of body composition and tissue development during cachexia implicates of a number of pathways and enzymes. The exact interacting factors, which regulate these enzymes, can be more closely examined during the disease progression.

Table 1. Comparision of body composition changes during different metabolic conditions in comparision to Cachexia (↑ - increase, ↓ - decrease, - little or no change, BW – body weight, BF – body fat, FI – food intake) – summation of effects discussed in the text

Condition	BW	FI	Protein Metabolism	Fat Metabolism	% BF
Anorexia	↓	↓	↓ synthesis ↓ proteolysis	↑ lipolysis ↓ lipogenesis	↓
CHF cachexia	↓	↓ or -	↓ synthesis ↑ proteolysis	↑ lipolysis ↓ lipogenesis ↑ Visceral fat deposit*	↓ ↑*
Cancer Cachexia	↓	↓	↓ synthesis ↑ proteolysis	↑ lipolysis ↓ lipogenesis	-
Sarcopenia	↓	↓ or -	↓ synthesis ↑ proteolysis	↑ lipolysis ↓ lipogenesis	↑
Obesity	↑	↑	↑ catabolism	↓ lipolysis ↑ lipogenesis	↑

***** In CHF cachexia regional differences in fat deposition may occur e.g., visceral fat deposit

1.9 Pathogenesis of Cachexia

Human epidemiology and experimental animal studies clearly show that the pathogenesis of cachexia is multifactorial and complex. Generally, research efforts have focussed on elucidating the role of hormonal factors or genes which influence weight loss or body compositional changes in cachexia.

The anorexia seen in cachexia cannot be solely explained by energy deficit. Other factors exist such as APRP initiated by IL-6 and IL-8 (interleukins), and induced by proteolysis inducing factor (PIF), a factor of tumour origin (Tisdale, 2003), all of which may contribute to the wasting process. (Tisdale, 2003) also notes that the energy deficit (increase in energy expenditure) may be partially due to increased UCP mRNA

expression. UCP-2 and UCP-3 expression in skeletal muscle are both upregulated in rodent models of cachexia, and may play a role in regulation of energy balance and lipid metabolism in cachexia (Gordon, et al., 2005). On the other hand, the loss of adipose and skeletal muscle tissues observed in cancer cachexia is primarily the result of increased lipolysis, decreased protein synthesis and increased protein degradation (via ATP-ubiquitin-dependent proteolytic pathway activated by PIF and TNF-α) (Tisdale, 2003).

At the enzymatic level, lipolysis in the fat cells of cachectic cancer patients was mainly attributed to the enzyme HSL (Agustsson, et al., 2007). While this provides a potential target for therapeutic treatment of cachexia, the occurrence of cachexia and the degree of wasting is known to differ between different cachectic conditions. Most cancer cachexia is seen in gastrointestinal cancers rather than in breast cancer, for instance (Tisdale, 2003). Depending on definition, the degree of cachexia may change, as (Anker & Rauchhaus, 1999) comments that the presence of documented non-intentional/ non-edematous weight loss (> 7.5%) of the premorbid normal weight, over a 6 month time period confers cachexia. Using this definition, 16% of an unselected CHF outpatient population was found to be cachectic (Anker & Rauchhaus, 1999). Thus, prognosis of cachexia changes with definition and is related to survival time. Studying dietary intake, resting energy expenditure (REE) and weight loss in 297 patients with generalized malignant disease, and their relation to survival, (Bosaeus, et al., 2002) showed that hypermetabolism and weight loss were significant predictors of morbidity. The factors which govern this survival time are thought to be divided into two main groupings; host derived factors (e.g., TNF-α, IL-1 and IL-6, IFN-γ, and leukaemia inhibitory factor, 2 –

pro-inflammatory cytokines), and tumour derived products with direct catabolic effect on host tissues (e.g., lipid mobilizing factor (LMF) and PIF) (Tisdale, 2003).

These factors regulate cachexia and may influence body weight through hypermetabolism. (Bosaeus, et al., 2002), observed that in 297 patients with generalized malignant disease, increased REE was not fully compensated for by increased food intake, and concluded that the feedback regulation of dietary intake in relation to energy expenditure is frequently lost in cancer patients. As mentioned, adipose tissue is affected during cachexia, particularly cancer cachexia, with loss of up to 85% of all adipose tissue due to the cachectic process (e.g., decreased lipogenesis) (Tisdale, 2003). This increased lipolysis may result from increased HSL, but also may be induced by increased β-adrenergic receptor activity with pro-cachectic cytokines, which are able to inhibit lipogenesis in adipose tissue (Tisdale, 2003). In cachexia there is a selective loss of skeletal muscle due mainly to decreased protein synthesis and increased protein degradation (Tisdale, 2003). In an experiment, with Sprague-Dawley rats by (Steffen, et al., 2008), cardiac cachexia was rapidly induced with monocrotaline. As a consequence, TNF action diminished by the use of a TNF receptor-1 or the general TNF production inhibitor (pentoxifylline) which, both attenuated body and skeletal muscle mass losses and reduced increases in selected ubiquitin proteasome pathway transcripts. Lastly, an acute cause of cachexia is fever, which increases thermogenesis, protein turnover, involving cytokine action and cachexia (Pi-Sunyer, 2000).

In summary, the pathogenesis of cachexia involves; pro-inflammatory factors which regulate lipolysis and protein synthesis/ degradation and indirectly by affecting

energy expenditure and food intake. These relationships have been studied in a plethora of animal and human studies as referenced in the following section.

1.10 Alterations in metabolism during Cachexia

1.10.1 Hypermetabolism and body compositional changes

Energy metabolism is largely governed through physiological systems, which regulate body weight, mitochondrial function and uncoupling of oxidative phosphorylation (Vidal-Puig, 2000). During disease state this energy homeostasis is altered, and the exact fundamental changes in the system which, give rise to changes not only in body weight, but also in body composition requires further understanding before treatment methods for cachexia or obesity can be mentioned.

Normally energy is stored as adipose tissue, but in cachexia, protein is metabolized to provide carbon skeletons for increased energy needs due to elevated energy metabolism. Thus, rapid weight loss ensues. During obesity the energy intake is exceeded and/ or expenditure diminished (Hosoda, et al., 1999), with higher body weight attributed to increased adipose tissue stores. Looking further into this weight regulation paradigm, cachexia and obesity either arise due to hyper-metabolism or hypo-metabolism at a mitochondrial level, respectively. Moreover, energy metabolism can be divided into energy producing (ATP) reactions (catabolism) or energy requiring (anabolism), with a possible third variable, proton leak (energy dissipation) (Rolfe & Brown, 1997). In addition, appetite and energy expenditure are under control of the hypothalamus through orexigenic peptides (e.g., NPY and MCH peptides) or anorexigenic peptides (e.g., Neurotensin, MSH, CRF) (Kalra, et al., 1999), providing the framework for regulation.

In a review by (Tisdale, 2003), the causes of weight loss are proposed to be due to increases in metabolism via either decreased energy intake, increased energy expenditure, or a combination of the two. During anorexia, caloric reduction contributes to lost adipose tissue, not lean body mass, whereas in cancer cachexia

there may be uniform loss of both kinds of tissue. In addition, skeletal muscle is wasted in cachexia with no change in visceral tissue, unlike changes observed in starvation. Observations of increased REE in patients with lung and pancreatic cancer, but not gastric and colorectal cancer, suggest that hypermetabolism may be regulated by different organs/ bodily regions. In all instances of cancer cachexia, there is weight loss. Further explanation suggests that regional changes in REE may not be reflected throughout the body. This is highlighted by the increases in UCP2 and UCP3 (mitochondrial proton leak) in skeletal muscle in cachexia (Tisdale, 2003).

Basal metabolic rate (BMR) (i.e., resting, stress free, no digestion at standard temperature), is identified as the steady state rate of heat production which, under normal conditions has a key role; determining normal energy intake for maintenance and growth (Rolfe & Brown, 1997; Rolfe, et al., 1999). Alteration of BMR during disease states may dictate the pathophysiology of the condition and also increase probability of mortality. Three main factors affect BMR; thyroid status, phylogeny and body mass (Porter & Brand, 1993). One of the contributing factors affecting metabolism is the regional site (e.g., cell type) of hypermetabolism, and plays an important role in the disease progression. Working in conjunction with this fact, the proportion of body weight represented by an individual tissue type is also a key factor. In rat, the skeletal muscle contributes 10-20% of BMR, yet accounts for 42% of body mass. The liver is approximately 2% of body mass, but contributes to 15-20% of total body oxygen consumption of the rat (Porter & Brand, 1993; Rolfe & Brown, 1997; Rolfe, et al., 1999). Some of this oxygen consumption is due to proton leak, with ROS (super-anion) production possibly stimulating this leak (~1-2% BMR) (Rolfe & Brown, 1997).

In the models of cachexia studied in this thesis, diminishing skeletal muscle due to muscle atrophy may influence decreases in energy expenditure, thus adding to a decline in appetite and food intake used for substrate oxidation. Thus, oxidisable substrates (i.e., carbohydrates, fatty acids, amino acids) provide a proton electrochemical gradient to produce ATP in the mitochondria, with excess substrate inhibiting substrate oxidation (Boss, et al., 2000). Hyperphagia (e.g., during diet induced obesity) increases standard metabolic rate by 25%, whereas fasting may lower it by 40% (Rolfe & Brown, 1997; Soboll, 1995), and thus proton leak is aligned to these changes (i.e., as an additional metabolic component) (Brookes, et al., 1998). This decrease in fasting (i.e., AngII induced weight loss due to satiety) may mask increases in energy expenditure due to the cachectic state.

In addition to physiological changes, BMR is also related to body size, and interestingly the BMR of the lizard is 20% less than the rat with the same body mass (Porter & Brand, 1993) as ectotherms (e.g. snakes and other reptiles) are a fifth less 'leaky' than endotherms (i.e., rats) (Brookes, et al., 1998). However, 20 – 40% of REE is used to counter proton leak down the electrochemical gradient across the inner mitochondrial membrane (Harper, 1997). Elevation in metabolic rate can far exceed calorie intake capacity and thus decreased food intake as a function of weight loss may contribute to the development of cachexia. Overall, even while receiving adequate calorie intake, most cachectic patients manage only to marginally maintain body weight or actually lose weight (Cariuk, et al., 1997), due to elevated protein catabolism and metabolic rate (Frayn, 1991). In this instance the body is in a state of hypermetabolism. Moreover, the daily energy expenditure and resting metabolic rate are greater in cancer than anorexic patients (Tisdale, 1991). This hypermetabolic state, is mirrored by a malnourished appearance as (Pasini, et al., 2003) suggest that a

quarter of CHF patients are malnourished, with two thirds also experiencing muscle atrophy.

Interestingly, hypermetabolism and protein catabolism may be interconnected, possibly through the role of pro-inflammatory cytokines. CHF patients with hypermetabolism, when considering their metabolic body size, also displayed increased leucine appearance rate (i.e., protein oxidation) (Toth & Matthews, 2006). Thus, there is a mechanistic link between hypermetabolism and muscle catabolism which deserves further investigation. Skeletal muscle apoptosis in heart failure patients is primarily caused by elevated ubiquitin in leg skeletal muscle with muscle atrophy possibly induced via elevated TNF-α signalling to skeletal muscle; further worsening ventricular dysfunction (Vescovo, et al., 2000), and energy homeostasis imbalance (King, et al., 1996b). During progression of CHF cachexia, there is altered catabolism and metabolism e.g. abnormal cytokine activation, and increased thyroid hormone or catecholamine levels (Anker, et al., 1997), which influence rates of metabolism and catabolism.

In summation, a broad body of knowledge points to energy homeostasis being governed by an elaborate system of regulation including basal metabolic rate, which is influenced by tissue type, body weight, pro-inflammatory cytokines levels and proton leak possibly via UCP's. Hypermetabolism and protein turnover when correlated with pro-inflammatory cytokines, may prove to be predictive measures for cachexia.

1.10.2 Role of Uncoupling proteins in Cachexia and Obesity

In general, the mitochondria are the governing body in relation to energy metabolism, particularly in the production of ATP. This system is driven by the trans-inner mitochondrial membrane protein - ATPase. UCP's are also inner mitochondrial membrane (IMM) bound proteins that function to regulate the transport of protons (H^+) from the outer to the inner mitochondrial matrix. Changes in the activity of UCP's, changes the membrane potential which is required for oxidative phosphorylation (via ATP-ase), and when dissipated without ATP production, leads to thermogenesis (e.g., the oxidation of a substrate in a futile cycle). The H^+ conductance of the IMM has two components; basal and augmentative. UCP2 and UCP3 belong to the augmentative components that cause H^+ leak, but do not catalyse basal leak (Stuart, et al., 1999), as they require fatty acids for H^+ transport (Brand, et al., 1999; Echtay, et al., 2001).

Some proposed biological roles for UCP's include regulation of proton leak across the IMM, thermogenesis and regulation of ROS production (Boss, et al., 2000). Another possible role for UCP's is modulation of free fatty acid (FFA) metabolism during fasting and as FFA transporters (Emre, et al., 2007; Negre-Salvayre, et al., 1997). The importance of fatty acid metabolism in obesity and heart failure may be explained in part by UCP's. Typically, a number of mitochondrial abnormalities lead to heart failure (e.g., elevated plasma free fatty acids, increased insulin resistance, tissue hypoxia), thus UCP's may affect progression of obesity (Murray, et al., 2007). This CHF-related proton leak may be due to the activity of UCP2 or UCP3, which are also increased during tumour growth and are associated with a concurrent elevation of TNF-α (Argiles, et al., 2003). They may be involved as regulators of energy homeostasis during cachexia.

Many studies have suggested a role for UCP's in elevated energy expenditure and weight loss in disease. Cachectic cancer patients (e.g. those with gastrointestinal adenocarcinoma) display elevated UCP3 mRNA expression in skeletal muscle (Bing et. al. 2001; Busquets et. al. 2005) but when compared to non-cachectic patients, no significant change in the UCP2 mRNA levels could be seen (Collins, et al., 2002). Recently, (Muscaritoli, et al., 2006), commented on research showing the action of LMF to increase UCP2 mRNA in skeletal muscle during cancer cachexia. Thus, there is a proposed role for UCP2 as a lipid metabolism regulator in cancer cachexia. This change in lipid metabolism may also effect changes that occur in food intake and may also be implicated in obesity development. The classic effect of over-expressing state IV respiration (i.e., Proton [H^+] leak without production of ATP), leads to an increase in muscle temperature (e.g., thermogenesis), with the over-expression of UCP3 mRNA possibly contributing to hyper-phagia, lower fasting glucose/ insulin and leading to obesity (Clapham, et al., 2000). The thermogenic effect in UCP1 and UCP2 is thought to be influenced via Ang II and adrenergic stimulation (Fukunaga, et al., 2000; Porter, et al., 2003).

UCP's are key regulators of metabolism homeostasis in obesity and cachexia. The alteration in expression of UCP's shows contrasting results from opposing disease and energy states. UCP2 is up-regulated in starvation and high fat diet (increased serum FFA) (Boss, et al., 2000) and UCP1 is decreased in consumption of a low fat diet (Rippe, et al., 2000). Similarly, UCP3 (skeletal muscle (SKM) specific) possess' roles in FFA metabolism, energy expenditure and body weight regulation (Fukunaga, et al., 2000; Kozak & Harper, 2000)

Further, membrane fatty acid composition may also influence oxygen consumption (Brookes, et al., 1998), thus dietary fatty acid composition may

influence uncoupling of oxidative phosphorylation and thus energy efficiency. Further, leptin decreases food intake possibly influencing lipolysis and initiation of UCP3 mRNA expression in muscle and decreased ROS generation (Boss, et al., 2000). Researchers have shown that hypertension prone rats display elevated UCP2 and UCP3 mRNA levels in cardiac-myocytes and skeletal muscle at 6 weeks of age, but reduced (70 and 36%) at 15 weeks, possibly via augmented sympathetic nerve activities paralleled with hypertension progression (Fukunaga, et al., 2000).

Examination of the interaction between cytokines and UCP revealed that TNF-α also increases UCP2 mRNA, and UCP2 mRNA is elevated during obesity (Merial, et al., 2000). Moreover, Ang II infusion significantly stimulates ROS production with UCP-2 reversing these effects (Park, et al., 2005). This implies a role for UCP's in chronic disease. UCP-2 may play a role in detoxification of free radicals (Russell, et al., 2002; Sanders & Tisdale, 2004) as per observations by (Guo, et al., 2006). These findings are important for understanding the complex relationship between body composition, and fat content; and the role that cytokine levels have in stimulating UCP's in obesity and cachexia. When combined, there is a growing evidence implicating UCP's in protection against ROS, particularly those generated during chronic illnesses such as cancer cachexia or heart failure, with their expression governed by alterations in substrate metabolism.

1.11 Changes in Carbohydrate, Lipid and Protein Metabolism

Cachexia and obesity are largely the result of alterations in the three related body composition components, namely carbohydrate, fat and protein metabolism.

1.11.1 Carbohydrate metabolism changes

Hypo-glycemia becomes increasingly prevalent during cancer cachexia (Costa, 1977). This hypo-glycaemia is due to tumour demand resulting in a 30% elevation in glucose turnover, 40% increase in hepatic gluconeogenesis and increased glucose synthesis from alanine and glycerol (Tisdale, 2000). This hypoglycaemia may also be caused by increased tissue glucose utilization, or decreased dietary intake (Beaufort-Krol, et al., 1999). Carbohydrate and fat fuel interactions affect eating behaviour, as a high carbohydrate diet suppresses fatty acid oxidation, thereby fostering fat storage (Friedman, 1998), limiting regulation of intracellular glucose metabolism, and insulin sensitivity (Frayn, 1991). Moreover, the cachectic patient suffers from insulin resistance, decreased lipogenesis and increases in both gluconeogenesis and glucose turnover (Urel, 1997). Lastly, it is well known that diet induced obesity results in fasting hyperglycaemia as observed by (Schemmel, et al., 1982).

1.11.2 Lipid metabolism changes

Adipose tissue is a major source of metabolic fuel (i.e., stored as triacylglycerol) and its subsequent lipolysis yields non-esterified fatty acids and glycerol. Both lipoprotein lipase (LPL) and HSL are involved in mobilization of stored fat energy. The extra cellular lipolysis is regulated by LPL and the intracellular lipolysis is regulated by HSL (Samra, 2000). The regulation of lipolysis may be

attributed to a number of hormones and lipolytic factors. Here I will mention a few important consequences to fat metabolism in cachexia and obesity. Ang II production has previously been linked to diet induced obesity, as renin deficient mice are leaner due to higher metabolic rate and decreased dietary fat absorption. This is reversed by Ang II infusion (Takahashi, et al., 2007). Moreover, white adipose tissue (WAT) influences energy homeostasis, glucose and lipid metabolism, vascular homeostasis and immune response. A high fat diet leads to hyperglycaemia and increased weight (Hosoda, et al., 1999). Interestingly, adipose tissue produces TNF-α, Ang II and interleukin-6, as well as anti-inflammatory molecules (i.e., adiponectin) (Sharma & Chetty, 2005), which may contribute to lipolysis.

In cachexia, lipolysis is increased due to an increase in host energy requirements causing mobilization of fatty acids and glycerol possibly attributable to lipid mobilizing factor (LMF) (Tisdale, 2000). There is further elevated FFA and glycerol turnover with an overall net loss of lipids from the host (Urel, 1997). Plasma nor-epinephrine concentrations have been found to be a predictor of elevated FFA oxidation (via elevated HSL) and whole body oxygen consumption (Lommi, et al., 1998). Obese rats display higher concentrations of circulating thyroxine, and lower proton leakage across the IMM (Ramsey, et al., 1996). The thyroxine accounts for a 75% increase in thermogenesis between hypothyroidism and euthyroidism, and a 50% increase between hypothyroidism to hyperthyroidism in the rat (Silva, 1999).

1.11.3 Protein metabolism changes

In both cachexia and obesity, protein metabolism is known to be governed by a number of pro-inflammatory hormones and proteolytic factors such as IL-6 and TNF-α. In particular, IL-6 is mentioned by (Inui & Meguid, 2003), to adversely influence (cause imbalance) orexigenic and anorexigenic pathways from the hypothalamus, which alters body weight regulation leading to either cachexia or obesity. During cachexia, catabolic mediators affect the body to result in increased whole-body protein turnover, decreased muscle protein synthesis and increased liver protein synthesis and muscle protein catabolism (Urel, 1997). Of notable difference between starvation versus cachexia, is that in the starvation state, changes in visceral organ metabolism occurs (e.g., liver and gastrointestinal tract); whereby these organs catabolise protein for the host energy use, while skeletal muscle is largely preserved. Further, protein synthesis and degradation, and protein turnover are largely reduced (Urel, 1997).

1.12 Catabolic Mediators of Cachexia

The human body's hormonal signalling plays a crucial role in the prognosis of the cachectic state. These physiological mechanisms are governed by an array of cytokines and tumour catabolic factors, involved in adipose and skeletal muscle tissue loss, notabily PIF, which is responsible for lean tissue loss (Argiles, et al., 1997a; Tisdale, 2000). Other pro-inflammatory cytokines/ factors include TNF-α, IL-1, IL-6, IF-γ, PIF, LMF, cortisol, insulin, and nor-epinephrine. These factors all mediate cachectic processes and cause long-term feeding inhibition (Argiles, et al., 1997b; Inui, 1999; Rubin, 2003) . It has been observered that the chronic overproduction of cytokines, (i.e., IL-1β, IL-6, and TNF-α) may influence the disease progression via

nuclear factor-KB and ATP-Ubiquitin-dependent proteolytic pathways. Cytokines may also interact with the central nervous system to alter the release and function of neuropeptides and melanocortin receptors in the hypothalamus, altering food intake and metabolic rate (Mak, 2007). Skeletal muscle proteolysis-inducing factors and arachidonic acid each successfully induce muscle proteolysis (Belizario, et al., 1991).

In addition to cytokines, some hormones (e.g., cortisol, glucagon and adrenalin) in humans produce cachectic features (Rubin, 2003). Further, Ang II is a key hormone in CHF pathophysiology and is also involved in catabolic pathways in cachexia (Brink, et al., 2001; McCarthy, 1999). The effects caused by pro-inflammatory cytokine imbalance can directly affect body composition, weight loss, reduced cellular nutrient supply and APRP production (e.g., TNF-α) (von Haehling, et al., 2007). In addition, pro-inflammatory cytokines often work in synergy or cascade to one another as both IL-6 and TNF-α inhibit food intake by acting directly on the brain to reduce reducing the release of the appetite stimulating peptide; NPY (von Haehling, et al., 2007).

The suppression of the profile of anabolic hormones promotes weight loss. The anabolic hormone ghrelin promotes the secretion of NPY (food intake), whilst insulin and leptin antagonises it (von Haehling, et al., 2007). Growth hormone is also increased in the presence of ghrelin. Growth hormone (GH) stimulates energy expenditure, causing a lipolytic effect and directly stimulates IGF-I release (von Haehling, et al., 2007). Previous investigators have shown that IGF-I is decreased during Ang II induced weight loss (Brink, et al., 1996), and its infusion leads to improved weight gain in cardiac cachexia (von Haehling, et al., 2007).

Overall, body compositional changes and to some degree energy homeostasis may be altered by the activities of pro-inflammatory cytokines or other metabolic

factors arising from tumour or tissue origin, which can dictate the disease prognosis and patient morbidity.

1.12.1 Role of anabolic factors in Cachexia and Obesity

Ghrelin, Growth hormone, NPY and insulin

Anabolic hormones are key regulators of cachexia development and may be used in treatment and recovery methods. The four main anabolic hormones are ghrelin, GH (Growth hormone), NPY and insulin. Ghrelin is a GH-releasing peptide, released by cells in the stomach and under hypothalamic control, may play a role in positive energy balance (e.g., stimulating food intake and inducing adiposity) (Nagaya, et al., 2001a). Nagaya, et al., (2001b), observed that in 74 patients with CHF, and especially those with cachexia (n = 28), plasma ghrelin and GH was significantly higher than in those CHF patients without cachexia. In addition, plasma ghrelin was correlated with the presence of TNF-α and negatively correlated with BMI (Body Mass Index). The authors concluded that ghrelin-induced positive energy effects (improved feed intake) may compensate under the imbalanced catabolic-anabolic relationship in cardiac cachexia (Nagaya, et al., 2001b). GH is mediated by IGF-I (Insulin-like Growth Factor), which are both anabolic hormones, essential for skeletal and myocardial growth and metabolic homeostasis (Nagaya, et al., 2001a).

Another important orexigenic hormone is NPY and it is the most widely distributed neurotransmitter in the mammalian brain (hypothalamus specifically). The roles of NPY are to stimulate feeding, decrease energy expenditure (e.g., via BAT (Brown Adipose Tissue) thermogenesis) and suppress powerful inhibitory signals for food intake and body adiposity (Inui, 1999). The primary physiological role of the NPY neurons in the hypothalamus is the re-establishment of energy balance and

adipose tissue stores after a period of negative energy balance (Inui, 1999). Another function of NPY is to block and reverse IL-1b-induced anorexia (e.g., cytokine-neuropeptide interactions) – with possible involvement of other orexigenic and/or anorexigenic signals in the anorexia and cachexia (Inui, 1999).

Insulin is another anabolic hormone which has an effect in cachexia, particularly in adipose tissue. In 138 patients with gastrointestinal malignancy, the provision of insulin treatment for > 3 months significantly stimulated carbohydrate intake, increased whole body fat and survival rate (Lundholm, et al., 2007). Higher serum insulin does not change IGF-I levels, and thus skeletal muscle is unaffected. It is noted that daily insulin treatment for > 3 months in catabolic cancer patients significantly improves micronutrient intake and increases net retention of body fat (Lundholm, et al., 2007).

In light of the action of anabolic hormone regulators of cachexia, they are under negative feedback and suppression by catabolic factors, which often dominate to control the outcome of disease progression and mortality.

1.12.2 Role of catabolic factors in Cachexia and Obesity

1.12.2.1 Leptin

Leptin is a hormone secreted by white adipose tissue. It modifies both orexigenic (appetite-stimulating) and anorexigenic (appetite-suppressing) molecules in the hypothalamus thereby controlling adipocyte energy stores (Inui & Meguid, 2003). When leptin receptors are blocked, this leads to obesity. Increased orexigenic and impaired anorexigenic signalling produces hyperphagia and obesity, while the reverse applies to anorexia-cachexia syndrome in which adaptive feeding response to starvation is lacking or insufficient (Inui & Meguid, 2003) Leptin also increases sympathetic nervous system activity, thermogenesis and energy expenditure (Engeli & Sharma, 2000), which all contribute to development of cachexia.

Leptin is elevated in CHF, especially in patients with severe exercise intolerance, and is correlated with TNF-α (Schulze, et al., 2003b). Other hormones secreted by WAT are adipsin, adiponectin, and resistin, which all have a role in obesity and cachexia (Guerre-Millo, 2002). Leptin release is linked to the decreased release of NPY. NPY interacts with anorexigenic (cholecystokinin, peptides YY and oxyntomodulin) and orexigenic (e.g., ghrelin) factors originating from the gastro-intestinal tract (GIT). These molecules regulate short-term food intake and growth hormone release. Impairment of this anabolic and catabolic balance may result in disorders of feeding behaviour and weight gain (obesity) or weight loss (cachexia) (Konturek, et al., 2004). Moreover, leptin and ghrelin oppose one another in appetite regulation. Leptin causes vasodilation and pro-inflammatory effects; contributing to cardiac cachexia and obesity-related cardiomyopathy, whereas ghrelin improves left ventricular function and cardiac cachexia in heart failure (Sharma & McNeill, 2005).

Most animal models of obesity, whether associated with genetic, diet-induced, or age-related obesity, display pronounced leptin resistance (Prima, et al., 2004). Ciliary neurotrophic factor (CNTF) has been recently used to induce leptin-like signalling pathways (e.g., weight reduction or severe cachexia), thereby circumventing leptin resistance (Prima, et al., 2004). Histamine, a central neurotransmitter is also an anorexigenic agent, which delays leptin resistance and accelerates lipolysis (Jorgensen, et al., 2007). Leptin is also involved in non-cachectic patients; linking metabolic, cardiovascular and respiratory abnormalities in CHF (Wolk, et al., 2003).

1.12.2.2 Acute Phase Response Proteins

The acute phase response protein (APRP) is a term which describes a collective number of proteins of hepatic origin, and are common manifestations of inflammation seen during cachexia. APRP are thought to result from the increased synthesis and release of cytokines (e.g., TNF, IL-1, IL-6) (Oldenburg, et al., 1993). Tissue inflammation is seen in cachexia and it invokes the acute phase response (APR), which encompasses physiological and metabolic changes (Tisdale, 2003). Hypoalbuminemia occurs due to the rapid synthesis of acute phase proteins (e.g., C-reactive protein (CRP), serum amyloid A protein, β2-macroglobulin and α-1-antitrypsin). The presence of the APR is correlated to weight loss and shortened survival time, as APR is mediated by pro-inflammation cytokines (e.g., TNF-α, ciliary neurotrophic factor (CNTF), IL-1 and IL-6etc.) (Tisdale, 2003). The blockage of IL-1 receptor has been demonstrated to attenuate weight loss and anorexia, however the receptors are dependent on IL-6 as passive immunization against IL-6 further reduces

hepatic APPR (e.g., decreased serum amyloid P and complement 3) (Oldenburg, et al., 1993).

In a study using 704 individuals with coronary heart disease it was observed that plasma levels of C-reactive protein and serum amyloid A protein were strongly correlated and inversely related to serum albumin. Interestingly, plasma C-reactive protein and serum amyloid A protein were also correlated with obesity and plasma lipids. It was concluded that inflammation was a causative factor of coronary heart disease (Danesh, et al., 1999). Lastly, it has been revealed that there is a link between inflammation (e.g., sialic acid measurement in serum) as a modulator of obesity and metabolic syndrome. Dietary n-3 PUFA is suggested to be beneficial in modulation of some degree of obesity disease risk via an anti-inflammatory mechanism (Browning, 2003).

1.12.2.3 TNF-α (Tumour Necrosis Factor)

TNF-α is a chief cytokine hormone, which is pivotal in regulating cachexia and obesity. The weight loss during cachexia is increased in the presence of increased levels of cytokines (i.e., TNF- α) and in decreased levels of insulin, glucagon, and glucocorticoid. In cachexia there is a decrease in white adipose tissue, decreased LPL, increased HSL (Hormone Sensitive Lipase) and inhibition of glucose transport to adipocytes. During obesity, there are opposing hormone levels as those mentioned in the previous sentence with both TNF- α and IL-1 increased. Both TNF- α and IL-1 regulate thermogenesis and influence adipocyte metabolism toward the catabolic side and their levels correlate to adipocyte mass, hypertension, diabetes and dyslipidemia (Cottam, et al., 2004). In addition, adipocyte hypertrophy may trigger adipocyte-

genesis, triggered by adipocytes producing IGF-I and binding proteins, TNF- α, and Ang II (Hausman, et al., 2001).

TNF-α causes metabolic acidosis, systolic and diastolic dysfunction with endo-toxin injection triggering depressed left ventricle function (Mann & Young, 1994) and indirectly suppresses human GH production (Anker, et al., 1997). TNF-α may induce weight loss in cachexia via uncoupling of mitochondrial respiration, increased skeletal muscle proteolysis, apoptosis, DNA fragmentation and increased levels of free and conjugated ubiquitin (Argiles, et al., 2003).

Once the individual is obese, TNF- α is released by adipocytes, which leads to anorexia and thus weight loss, (reversing obesity) via increased thermogenesis, causing insulin resistance, impaired glucose output and decreased Glucose Transport Protein 4 (GLUT-4) (Argiles, et al., 1997b)**.** Further, TNF-α mitigates obesity via counteracting excess food intake. This is thought to compensate for elevation of thermogenesis via increased sympathetic activity, causing lipolysis and decreased lipogenesis. Similarly, in Cachexia, TNF-α induces anorexia, increased thermogenesis and an adipocyte lytic effect (Argiles, et al., 1997b)**.**

Others have observed weaker correlations in relation to systemic TNF-α (Saarinen, et al., 1990). TNF-α may mediate systemic changes through IL-1 uncoupling of β-adrenoreceptors in CHF (Mann & Young, 1994). Further, it is thought to be elevated by stimulation of nitric oxide synthase in myocardium (Mann & Young, 1994) of the failing heart (i.e., causing decreased left ventricle function, structural modifications and cachexia) (Anker, et al., 1997; Mann & Young, 1994). Another pro-inflammatory cytokine IFN-γ has biological activities which overlap TNF-α, which can be nullified using monoclonal antibodies against IFN-γ (Argiles, et al., 2003). Lastly, IL-15 (a cytokine highly-expressed in skeletal muscle), TNF- α, and

leptin could all play a decisive role as hormonal messages between adipose tissue and skeletal muscle (Argiles, et al., 2005).

1.12.2.4 IL-1 and IL-6 (Interleukins)

Interleukins are another cytokine group involved in the initiation of cachexia. Nude mice implanted with MCF-7IL-1 cells who develop cachexia, experience 'cross talk' between the epithelial and stromal cells. This 'cross talk' is thought to be mediated through IL-1, with the degree of IL-1 release correlated with cachexia. It was suggested that IL-1 increases leptin expression in stromal cells, which principally targeted lipid metabolism (Kumar, et al., 2003).

Another IL, IL-6 is associated with prostrate cachectic patients (Kuroda, et al., 2007). Overall, the presence of IL-6 (>7pg/mL in serum) was strongly correlated to higher degree of mortality. In addition, these patients also had lower serum total protein, albumin, and cholesterol levels, haemoglobin levels, and body mass index. The authors concluded that IL-6 is one of the numerous factors which contribute to the complex syndrome of cancer cachexia.

Cachexia as a disease is promoted by pro-inflammatory cytokines, in particular, IL1 and IL6. In 21 elderly people with protein and energy malnutrition in comparison to 22 subjects matched for BMI of ~25 were studied over a 3 month period. Isolated monocytes were stimulated with lipopolysaccharide (LPS) and IL-6 was found to be higher in the malnourished subjects than in the control subjects. There was a higher generation of IL-l and IL-6 in patients with protein and energy malnutrition relative to the control subjects (Cederholm, et al., 1997). The authors concluded that there is an enhanced generation of proinflammatory cytokines (i.e.,IL-1 and IL-6) in elderly malnourished patients. These series of findings supported the

author's hypothesis that protein and energy malnutrition during disease is associated with an activation of the inflammatory system, via inflammatory cytokines (e.g., IL-l, TNF-α, and IL-6), which are involved in the pathogenesis of the cachexia (Cederholm, et al., 1997). There is an interrelating role between cytokines, particularly IL-1 and TNF-α. Both are associated with elevated REE, satiety, and decreased body weight. They are also linked with APRP synthesis, induce systemic immune response (e.g., antibody formation) involving cancer-anorexia via elevated corticotrophin-releasing hormone (Argiles, et al., 2003; Little, 1991; Roubenoff, 2004). In addition, a majority of the patients studied also suffered from pulmonary disease and thus hypoxia may have been one mechanism by which to activate the immune system (Cederholm, et al., 1997). Moreover, the authors also noted that during most inflammatory disorders, the generation of proinflammatory cytokines is balanced by production of anti-inflammatory cytokines (e.g., IL-lra, IL- 10, and TGF 1) (Cederholm, et al., 1997) as a means of counter-regulation.

1.12.2.5 LMF (Lipid Mobilizing Factor)

Fat metabolism during cancer cachexia is one of the key tissues affected. This is the result of increased lipolysis, reduced lipogenesis, increased hepatic secretion of VLDL (very low density lipoprotein), increased *de novo* fatty acid synthesis (i.e., turnover) and a futile cycle of fatty acids between liver, tumour and adipose tissue (Tisdale, 2004). Another factor altering fat metabolism is deregulation of lipid synthesis due to HSL activation (Tisdale, 2004). There exists a novel urinary protein (LMF) which has a molecular weight of M_r 43000 and is excreted in the urine of cancer cachexia (McDevitt, et al., 1995). LMF is a tumour-derived hormone, which appears to stimulate lipolysis in isolated murine epididymal adipocytes, and has an

amino acid sequence homologous with human Zn-α_2-glycoprotein (Hirai, et al., 1997).

The presence of LMF is directly proportional to tumour mass and liver glycogen depletion (Todorov, et al., 1998). There also exists other urine and serum factors identical to cachexia inducing factors, which act to depress protein synthesis and elevate prostaglandin E_2 ,which have been detected in mice bearing the MAC16 (Murine colon adenocarcinoma) (Belizario, et al., 1991; Smith & Tisdale, 1993). In this context, there may be an array of tumour, host-derived factors which act in synergy to modulate lipid metabolism in cancer cachexia and other diseases.

1.12.2.6 PIF (Proteolysis Inducing Factor)

PIF is a novel glyco-protein (24,000 Da) excreted in the urine of cancer patients with cachexia (e.g., pancreatic, breast, lung) (weight loss >1.5kg per month), but not in the urine of non-cachectic cancer anorexic patients (Cariuk, et al., 1997). PIF is observed in a range of tumour types (e.g., MAC16 colonic adenocarcinoma, G361 melanoma) but not other conditions (e.g., surgery, sepsis, burns etc.), and further not associated with serum C-reactive protein concentration (i.e., inflammation response) (Cariuk, et al., 1997; Lorite, et al., 1998; Todorov, et al., 1998; Wigmore, et al., 2000). PIF is thought to undergo renal metabolism alterations (Gullu & Marangoz, 1999), and is stored in tumour cells (Cabal-Manzano, et al., 2001).

PIF induces proteolysis directly *in vivo* (via injection), causing elevated FFA, glucose, ubiquitin and *in vitro.* It does not resemble any known cytokines, as its functional group resides in the carbohydrate moiety (Cariuk, et al., 1997; Tisdale, 2000). PIF also causes significant loss of spleen, soleus and gastrocnemius muscles, and increased liver weight has been observed (Lorite, et al., 1998). PIF further

depresses protein synthesis, increases protein degradation in C_2C_{12} myoblasts and myotubes (Gomes-Marcondes, et al., 2002; Whitehouse & Tisdale, 2003). PIF is associated with enhanced prostaglandin E_2 (PGE_2) release (i.e., inhibited by EPA supplementation, monoclonal antibodies to PIF and EPA, and cytokine anti-sera) (Cariuk, et al., 1997; McCarthy, 1999), via EPA incorporation into muscle phospholipids (Smith & Tisdale, 1993).

Similarly, another catabolic factor identified is Azaftig (24kDa proteoglycan), is present in urine of AIDS patients. Azaftig causes selective depletion of adipose tissue, and arises from phagocytic cells (Figueroa, et al., 1999). As mentioned, other catabolic factors exist in conditions other than cancer or AIDS (i.e., sepsis cachexia - (Goldberg, et al., 1988), such as the cyto-toxic hormone from tumour periphery (4,200 – 6200Da) which was observed to depress liver catalase in mice and amino acid incorporation into proteins, and acts in an immuno-suppressive capacity (Rubin, 2003). Moreover, Trehalose dimycolate is a by-product of mycobacterium metabolism of *Nocardia asteroides*, which has a toxic effect causing cachexia and death, 30 days after the initial injection (10μg per day) (Silva, et al., 1988). Lastly, there is yet to be an identified factor, existing in cardiac cachexia, which induces rapid weight loss, and thus may prove to be an exciting area of study.

1.13 Role of Angiotensin II in CHF Cachexia and Obesity

Angiotensin II is a key molecule in the renin-Angiotensin system. Ang II is responsible for changes in blood pressure and sodium/ fluid retention (e.g., thirst, sweat, urine). Upon systemic infusion, Ang II causes weight loss, adipocyte proliferation and thermogenesis (Hussain, 2003; Porter & Brand, 1993). Ang II links both cachexia and obesity development, mainly through alterations in fat metabolism. Advanced heart failure is characterized by increased activation of the renin-Angiotensin system and the development of cachexia (Cabassi, et al., 2005). Increased Ang II modulates lipid metabolism, by increasing interstitial glycerol and nor-epinephrine levels, resulting in decreased sub-cutaneous and visceral fat (Cabassi, et al., 2005). Further, Ang II is up-regulated in CHF, via a pressor-independent mechanism with separate metabolic effects, inducing Cachexia which is independent of anorexia (Brink, et al., 1996).

The principle prognosis in cardiac cachectic patients is increased activity of the Angiotensin system and IGF-I. In this instance, Ang II reduces circulating and skeletal muscle IGF-I yet increases cardiac muscle IGF-I. This in turn elevates the ubiquitin-proteasome pathway, and apoptosis (Delafontaine & Akao, 2006). It has been noted that caspase-3 may act in this pathway to induce skeletal muscle proteolysis which may lead to actin cleavage and increased apoptosis (Song, et al., 2005). Moreover, Ang II infusion markedly increases protein degradation via inhibition of the autocrine IGF-I system (Brink, et al., 2001). Age also alters the degree of Ang II-linked proteolysis. Interestingly, Ang II limits growth in young rats and induces cachexia in older rats by progressive muscle atrophy and lipolytic effects (Brink, et al., 2001; Schulze & Spate, 2005). Decreased IGF-I is also observed in pair

fed controls, confirming the primary effect of Ang II infusion being reduced feed intake, and increased kidney and left ventricular weights (Brink, et al., 2001). In all, these effects lead to cardiac cachexia, particularly proteolysis.

Growth hormone and IGF-I have been observed to have beneficial effects on myocardial function in animal models of heart failure including vasodilatation, stimulation of cardiac hypertrophy and prevention of apoptosis). ACE inhibitors and Ang II receptor blockers reduce the risk of developing type 2 diabetes mellitus, and decrease both ROS production and β- cell dysfunction (Leiter & Lewanczuk, 2005). This leads to improved insulin sensitivity compared to fructose fed control rats (Higashiura, et al., 2000).

Like cardiac cachexia, obesity is a result of body weight dysfunction, and is caused by an excessive energy intake or decreased energy expenditure. This metabolic imbalance may be caused by hyper-insulinemia linked with obesity which may accelerate cardiac structural damage via interacting with Ang II and increasing collagen production and deposition (Kramer, 2006). (Porter, et al., 2003) infused Ang II systemically and found decreases in body weight and food intake. The decreased body weight was thought to be caused by increased energy expenditure (i.e., thermogenesis, increased UCP1 mRNA expression in Brown Adipose Tissue). Moreover, (Porter & Potratz, 2004) also observed transient decrease in food intake. They observed that following cessation of AngII infusion, the rat body weight continued to decrease most likely due to increased energy expenditure (i.e., thermogenesis; UCP1). It is thought that Ang II acts directly on the brain (possibly the AT1 receptor protein) (Porter, 1999) to affect food intake and energy expenditure in a manner not related to water intake. In combination, obesity, cardiac failure and

subsequent cardiac cachexia represent significant contributors to mortality linked by Ang II.

Weight loss attributable to Ang II infusion could also be caused by elevation of pro-inflammatory cytokines in the myocardium and plasma (i.e., Interleukin 1, interleukin 6 and TNF-α). Paulus (2000) correlates the presence of cytokines and induction of striated muscle mass wasting and cachexia, possibly through elevated ubiquitin and macrophage activation (Peterson, et al., 2006). In particular, ghrelin may be a hormone of intense study in future use of this animal model of cardiac cachexia as it regulates positive energy balance via stimulating food intake and inducing adiposity (Nagaya et. al. 2001) and be used to treat anorexia-cachexia syndrome.

1.14 Role of Ang II in Obesity and oxidative stress

Adipose tissue is an important source of Angiotensinogen; a precursor of Ang II. Ang II stimulates adipocyte production and the adipocyte release of insulin and fatty acids (Cassis, 2000). (Porter, et al., 2003) notes that infusion of Ang II causes body weight loss and satiety, thus Ang II may have a negative feedback regulating diet induced obesity and fat accumulation. Contrasting this hypothesis, hyperphagia in rodents induces local formation of Ang II via Angiotensinogen secretion from adipocytes, promoting adipocyte growth and preadipocyte recruitment (Engeli, et al., 2003). Thus, Ang II is implicated in development of insulin resistance via interaction with adipose tissue (Sharma, 2004).

Ang II also is a key regulator of oxidative stress. Hypertension, endothelial dysfunction and insulin resistance are associated conditions that share oxidative stress and vascular inflammation as common features. Adiponectin is a modulator of lipid metabolism and exerts a potent anti-inflammatory activity. Adiponectin is decreased during Ang II-induced oxidative stress (Hattori, et al., 2005), and in the presence of increased nitric oxide and super oxygen dismutase (Chabrashvili, et al., 2003). Oxidative stress in blood vessels can be triggered by an array of vasoconstrictor mechanisms, (e.g., blockade of nitric oxide synthase). This effect causes vasoconstriction via bioinactivation of nitric oxide, and by nitric oxide synthase independent mechanisms (Wilcox, 2002). Paracrine and hormonal factors released from adipocytes implicate nitric oxide in the pathophysiology of obesity-induced hypertension. Results from these studies show that Ang II is pivotal in the induction of oxidative stress. Oxidative stress may be alleviated by dietary intake of antioxidants (e.g., green tea catechins) and prove useful in cachexia treatment.

1.15 Animal models of Cachexia

A wide array of animal models have been developed to investigate cancer cachexia. However, few animal models have examined CHF cachexia. In general the studies can be divided into those dealing with hormonal levels, alterations in energy expenditure or nutritional treatment of cardiac cachexia. (Schulze, et al., 2003a) remarked that CHF is associated with metabolic abnormalities leading to a catabolic syndrome in advanced stages of the disease (i.e.,increased IGF-I). Decreased local expression of IGF-I in CHF animals correlates with decreased muscle fibre cross-sectional area (e.g., muscle hypotrophy). Other models, using Ang II infusion, induce CHF have revealed that Ang II increases the rate of nitric oxide synthesis, increase blood pressure, abdominal temperature, and decreases WAT, body weight, leptin and circulating IGF-I (Brink, et al., 2001; Cassis, et al., 1998; Schulze & Spate, 2005). Further work identified that the origin of elevated energy expenditure is attributable to UCP1 and thermogenesis (Porter, et al., 2003).

In an attempt to develop treatments for weight loss in CHF, (Nagaya, et al., 2001b) developed a rat model using left coronary artery ligation to induce CHF cachexia. They infused rat ghrelin, to increased serum GH and IGF-I. The rats treated with ghrelin proceeded to gain weight, while experiencing higher cardiac output, inhibition of LV enlargement, and increased LV fractional shortening compared to control CHF rats. Further, ghrelin attenuates body weight loss in CHF cachexia, while improving cardiac structure and function (Nagaya, et al., 2001b). Ghrelin has also been noted to improve insulin resistance, particularly under a high fat diet (Asakawa, et al., 2003). The development of cardiac cachectic models as distinct from CHF is of great importance to successfully characterize and treat this disease.

1.16 Animal models of Right Ventricular Pacing (RVP)

Numerous large animal (e.g., dog, sheep, and swine models) of CHF have been developed, but few investigate cardiac cachexia. These CHF models imitate the combined interactions of cardiac dysfunction, neuro-hormonal processes and peripheral vascular abnormalities, either in naturally occurring CHF or by using interventions to induce CHF (i.e., tachycardia), leading to a clinical profile similar to human HF (Power & Tonkin, 1999). Heart failure is mainly due to damage to ventricular myocardium (e.g., by chronic ischemia) (Power & Tonkin, 1999). As heart failure is characterized by systolic and diastolic dysfunction, after two and a half weeks of pacing there is a significant increase in heart rate and left ventricular end-diastolic pressure and systolic wall thickening (Neumann, et al., 1999). Pacing is also associated with increased plasma nitrate levels, nitric oxide production and plasma creatinine (Bernstein, et al., 1997).

Ovine hearts are similar to adult human in size and in venous blood drainage but lack an excessive degree of collateral circulation as found in the dog. The small heart size of the pig is similar to the human (Power & Tonkin, 1999). Traditionally, HF is induced via pacing using endocardial or epicardial electrodes at 220 - 250 bpm (beats per minute) and an end-stage in 1 month. This method rapidly decreases cardiac contractile function via depression of LV and cardiac output. Further, HF leads to alterations in cardiac morphology, thinning of ventricular myocardium, atrial dilatation and cell hypotrophy (i.e., elongation) (Power & Tonkin, 1999), using modified rapid pacing at 170 – 190 bpm. The canine and ovine heart mimics LV dysfunction in human patients. Once a model is characterized, treatment can be tested *in vivo.*

1.17 Treatment of Cachexia

1.17.1 Exercise

Regular exercise can mitigate muscle protein wasting, and prevent alteration of mitochondrial and cytosolic proteins (enzyme activities). Progressive resistance exercise training predominantly increases contractile protein mass via increased muscle protein synthetic rate more than muscle proteolysis. The muscle amino acid balance is increased for up to 2 days after exercise training (Zinna & Yarasheski, 2003). Alteration of the dietary arginine-methionine balance by the use of synthetic L-amino acids inhibits tumour growth, maintaining body weight in cancer cachexia (Millis, et al., 1998). Resistance training may prove useful (Ardies, 2002) with insulin treatment reducing insulin resistance, preserving lean mass, as do semi-synthetic progestational steroids.

1.17.2 Dietary

In an attempt to alleviate cachexia, a number of dietary strategies have been employed (e.g. oral amino acid ingestion stimulates influx of amino acids into myocytes and muscle synthesis). The BCAA (Branched Chain Amino Acids) (leucine, isoleucine and valine) in particular provide amino groups for protein and carbon skeletons for skeletal muscle energy requirement (Busquets, et al., 2000), thereby increasing body weight and whole body protein turnover (Tayek, et al., 1986) and ↓protein degradation (von Haehling, et al., 2007). Several factors contribute to altered amino acid metabolism in obesity and cachexia including modulation of dietary intake, increase in BCAA oxidation, net catabolism, reduced protein synthesis and proteolysis. The latter is possibly activated by tumour or host-derived mediators causing alterations in amino acid demand and metabolism (Baracos & Mackenzie,

2006). Lastly, the use of GH supplementation promotes muscle weight gain in tumour-bearing animals (Bartlett, et al., 1995).

Amino acids are partially oxidized in the liver, converted to glucose, liberating ATP needed for gluconeogenesis. This oxidation accounts for one half of daily liver oxygen consumption (Jungas, et al., 1992). Surplus amino acid catabolism limits use of other substrates for heat production, therefore it does not impact on metabolic rate (MacLeod, 1997). Decreased fat-free mass causes decreased muscle amino acid due to efflux from the muscle (Engelen, et al., 2001). CHF patients release taurine from muscle tissue, whereas control patients have a net uptake of glutamic acid and taurine (Aquilani, et al., 2005). Exercise in CHF patients releases significant amounts of phenylalanine, BCAAs; histidine, alanine, but lowers glutamine and taurine uptake via alterations in intermediary and energy metabolism within myocytes (Aquilani, et al., 2005).

Cachexia stimulates the consumption of large amounts of energy and amino acids obtained via skeletal muscle catabolism with worsening prognosis leading to morbidity and increased elevated energy expenditure (Tisdale, 2002). However, artificial nutrient supplementation has been observed to be ineffective (Bozzetti, et al., 1999) as hyper caloric feeding is thought to induce weight gain primarily as fat deposition.

1.17.3 Anabolic hormones

In some instances, nutritional intervention of cachexia using a high calorie or high - protein supplement fails to correct energy or protein imbalance (Tisdale, 2004; Wigmore, et al., 2000). Satiety plays a major role in decreased food, and thus energy, intake. In carcinomas of the gastrointestinal region, satiety may be a physical, not neurohormonal, obstacle to resumption of appetite. The synthetic prostaglandins (e.g., medroxyprogesterone acetate) may be used to improve appetite and weight gain (Simons, 1998), increasing energy intake of protein, fat, and carbohydrates, but also increasing fat mass which is correlated to energy intake and hypothalamic synthesis of NPY (Simons, 1998). The blockage of type 3 serotonergic receptors by MPA can improve anorexia in cancer-affected animals by stimulating appetite and influence satiety (Edelman, et al., 1999), and improvement in protein deposition (Edelman, et al., 1999; Frayn, 1991). Meloxican, (i.e., a cyclooxygenase-2 inhibitor) suppresses MAC16 tumour growth and, combined with the actions of PIF, completely suppresses tumour growth (Hirai, et al., 1997; Hussey & Tisdale, 2000) .

Growth Hormone Receptor Protein (GHRP) improves LV function and cardiac remodelling in CHF rats and inhibits CHF Cachexia development, (i.e., increases body weight and suppreses cardio-myocyte apoptosis) (Xu, et al., 2005). Interestingly, cancer cachexia, can be suppressed via supplement with increased carbohydrate calories and EPA and DHA (Docosahexaenoic Acid) which antagonize weight loss and tumour growth (Tisdale, 1991)**.** Further, the addition of amino acid supplementation assists to elevate protein synthesis in cancer cachexia (Bozzetti, et al., 2000). Moreover, fish oil contains EPA and DHA, and this supplementation significantly increases the proportion of total n-3 PUFA’s in ventricular membrane phospholipids compared with saturated fats (Leifert, et al., 2000). Thus

supplementation of EPA and DHA stabilizes weight in pancreatic cancer via inhibition of the ubiquitin proteasome pathway (Tisdale, 2004). This inturn increases protein intake, decreases REE, and attenuates PIF induced muscle catabolism (Barber, et al., 1999; Cariuk, et al., 1997).

Other compounds, (e.g. Clenbuterol (β2-adrenoceptor agonist)), can treat mice which suffer from dystrophic muscle degeneration, thus opposing muscle weakness and hypotrophy (Zeman, et al., 2000). Further, human GH has been used to increase muscle mass (i.e., elevated energy expenditure and fat oxidation), and thus may be a good candidates for cachexia treatment (Lange, et al., 2000). Anti-cytokine treatment (e.g., anti-TNF-α) may be another successful treatment method (Morley, et al., 2006).

1.18 Diet induced Obesity, Reactive Oxygen Species and Antioxidants

Obesity alone is the cause of 11% of cases of cardiac failure in men and 14% of cases in women in the United States (Galinier, et al., 2005). Being overweight or obese has become highly prevalent in Western countries and is rapidly reaching epidemic proportions in the developing world (Sharma & Chetty, 2005) and is linked to mild hypertension (>158mm Hg) and oxidative stress (Dobrian, et al., 2001). Thus, a link exists between obesity, CHF and possibily cardiac cachexia. Obese patients shunt lipid into the skeletal muscle, liver and pancreatic β-cells with the degree of lipid infiltration into skeletal muscle and liver highly correlated to insulin resistance and adipocyte hypertrophy (Heilbronn, et al., 2004). Preventative treatment appears to be the most appealing method to control these series of diseases.

Antioxidants (i.e., reducing agents also known as ‘thiols’ or ‘polyphenols’) impede oxidation and protect against cardiovascular disease (Lam, et al., 2007).

Oxidation reactions (e.g., respiration) are pivotal for life but may also be damaging. Reactive Oxygen Specoes (ROS) and reactive nitrogen species, include hydrogen peroxide (H_2O_2), hydroxyl radical (·OH), superoxide anion (O_2^-), nitric oxide (NO*) which are all beneficial (eg. fight infectious agents, cell signalling in tumour cells - apoptosis) and also detrimental (e.g., damage to lipids, membranes, proteins and DNA) to health (Valko, et al., 2007).

Examples of antioxidants are glutathione, vitamin C, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidises with their absence causing inhibition of the antioxidant enzymes, oxidative stress and finally apoptosis (Vertuani, et al., 2004). Organisms have a complex network of antioxidant metabolites and enzymes that prevent in unison oxidative damage to cells (i.e., DNA, proteins and lipids damage) (Vertuani, et al., 2004). Other antioxidants are found in numerous beverages and foods (e.g., cocoa, coffee, green tea, apples, berries).

Lastly, feeding of a high fat diet to mice induces hyper-glycemia, hyper-insulinemia and obesity (i.e., palm oil diet) with a fish oil diet mediating decreased body weight and increased proton influx in rat liver mitochondria. A major cause of obesity associated insulin resistance is the high fat (saturated) content of the western diet (Ikemoto, et al., 1996). As already discussed, fish oil also alleviates tumour growth and cachexia, in cancer cachexia and possibly obesity as well.

1.19 The role of anti-oxidants in the treatment of diet induced Obesity

Obesity is associated with oxidative stress and mitochondrial ROS dysfunction. In a study by (Dong, et al., 2007), they investigated the effect that metallothionein (scavenger of free radicals) had in a high-fat diet (> 3 months) induced myocardial and mitochondrial dysfunction. The high-fat diet mice displayed increased ROS production (i.e., western blot analysis – enhanced phosphorylation of nuclear factor Foxo3a without changes in Foxo3a, Foxo1a, pFoxo1a, whereas these changes were negated by metallothionein, with the exception of pFoxo3a. Thus, metallothionein may protect against high-fat diet–induced cardiac dysfunction possibly via associated with up regulation of PGC-1 and preservation of increased mitochondrial integrity. In a study by (Yuan, et al., 2001), the researchers observed that the delivery of high doses of salicylates reversed hyperglycaemia, hyper insulinemia, and dyslipidemia in obese rodents by sensitizing insulin signalling. (Yuan, et al., 2001), over-expressed IB kinase (IKK) attenuated insulin signalling in cultured cells, whereas IKK inhibition reversed insulin resistance. These authors concluded that the inflammatory process in the pathogenesis of insulin resistance in obesity could be attributed to the IKK pathway as a target for insulin sensitization.

1.19.1 Cocoa and Obesity

Cocoa (*Theobroma cacao*) is rich in specific antioxidants, (i.e., polymer procyanidin), similar to those found in vegetables and tea. Antioxidants may defend against ROS formation, oxidation of LDL-cholesterol and support heart disease protection (Weisburger, 2001). The cocoa bean contains ~0.2% caffeine with the main methylxanthine compound in cocoa being theobromine ~ 2.5% by weight (Langley-

Evans, 2000). Theobromine has potent anti-oxidant and anti-bacterial effects (Matsui, et al., 2005). Cocoa intake has been noted to reduce cholesterol, fatty acid bio-synthesis. Cocoa ingestion with a high fat diet decreases weight gain in mice (Matsui, et al., 2005). Further, plasma leptin concentration and white mesenteric adipose tissue are also decreased, possibly via UCP2 thermogenesis (Matsui, et al., 2005). This effect may be the synergistic action of both procyanidin and caffeine. Moreover, cocoa ingestion has also been observed to increases bone density and bone strength in older woman (Hodgson, et al., 2008), and thus may be used as a nutritional supplement in suffers of gender and age related osteoporosis.

1.19.2 Coffee, caffeine and Obesity

Coffee is another commonly consumed beverage worldwide and also contains caffeine and anti-oxidants like cocoa and green tea. A recent hypothesis suggests there are 28 different coffee odorants which may be beneficial to stimulate appetite (Dorri, et al., 2007). In further supporting the role of caffeine in weight loss, (Cheung, et al., 1988) observed that chronic caffeine treatment increased lipolysis in rat epididymal adipose tissue. This led to decreased body weight, epididymal fat pad weight and mean adipocyte diameter when compared with control rats. However, other researchers; (Bracco, et al., 1995) failed to observe an effect of thermogenesis and coffee consumption in obese subjects. The degree of bean roasting may influence the presence and amount of caffeine or type of anti-oxidants present in the coffee, thus explaining the degree of variation between studies concerning obesity treatment.

Observations of postmenopausal woman with high caffeine intakes have displayed significantly higher rates of spinal bone loss (>300 mg/d) (Harris & Dawson-Hughes, 1994; Rapuri, et al., 2001) . However, other researchers (Conlisk &

Galuska, 2000) hypothesized that caffeine intake was not a significant predictor of bone mineral density (BMD) after adjusting with linear regression models for potential confounders (i.e., height, BMI, age, calcium and protein intake, alcohol and tobacco use).

(Denaro, et al., 1991) observed that increasing caffeine dosage (i.e., coffee) increased the 24-h area under the curve (AUC) for total sum of FFA. Further, there is significant increase in plasma FFA (i.e., lipolysis) in habitual coffee drinkers compared with a control group of non-coffee and decaffeinated-coffee drinkers (Cocchi, et al., 1983). The anti-oxidant compounds in coffee may also influence immune function and disease outcomes. High consumption of caffeine-containing coffee is associated with higher adiponectin and lower inflammatory marker concentrations (Williams, 2008).

Caffeine is found in green tea, coffee and its metabolites in cocoa. Caffeine stimulates body fat reduction in a dose dependent manner (0.025 – 0.1%) in rats fed a high fat diet. This effect is caused via increased catecholamine (epinephrine, norepinephrine and dopamine) and free fatty acids (Kobayashi-Hattori, et al., 2005). Lipolysis is induced via elevated catecholamines, leading to stimulation of the sympathetic nervous system (Kobayashi-Hattori, et al., 2005). Lastly, it has been observed that caffeine increases noradrenaline and the associated increase in lipolysis in adipose tissue cells via HSL (Han, et al., 1999).

1.19.3 Green tea and Obesity

An alternative approach to hypo-caloric diet is addition of antioxidants to decrease body weight with an iso or hyper-caloric diet. In this thesis, green tea (*Thea sinensis*) is used successfully as green tea possesses the monomeric epigallocatechin gallate (EGCG), theaflavin, polymeric thearubigins, which are polyphenols, and is one of only 17 Chinese medicinal herbs which display inhibition of fatty acid synthase (Tian, et al., 2004). Other catechins include epigallocatechin, epicatechin and epicatechin gallate (Wolfram, et al., 2005). Langley-Evans (2000) found that green tea infusates possessed approximately 2.5-fold greater antioxidant capacity than both types of black tea infusates. Intravenous injection of EGCG (catechin found in green tea) induces weight loss in rats (Wolfram, et al., 2005). Injection of pure green tea catechins (i.e., EGCG) intraperitoneal acted to reduce food intake, body weight and decreasing the following hormones levels in blood; leptin, insulin, IGF-I, glucose and triglyceride (Kao, et al., 2000). The researchers concluded that that the effect of EGCG was independent of an intact leptin receptor and that EGCG interacts directly with a section of a leptin-independent appetite control pathway (Kao, et al., 2000). Lastly, ingestion of TEAVIGO, a green tea extract, reduces blood pressure, type II diabetes, and supports weight loss (Wolfram, et al., 2005).

As a drink, green tea consumption provides a multitude of benefits including antiangiogenic, anti-carcinogenic, increases energy expenditure, fat oxidation and antioxidant activities (i.e., polyphenols; catechins), especially oolong tea (Han, et al., 1999; Wolfram, et al., 2005). Further, consumption of green tea has been linked to pancreatic lipase activity inhibition and lipid peroxidation inhibition. This is mainly due to tea saponin fractions in oolong tea; distinct from caffeine and tannins (Han, et al., 1999). The galloyl moiety of the green tea catechin suppresses postprandial

hypertriacylglycerolemia by slowing down triacylglycerol absorption through the inhibition of pancreatic lipase (Ikeda, et al., 2005).

Green tea extract (containing 25% catechins) induces totals digestive and pancreatic lipase inhibition at 80mg/g dosage. This inhibits fat digestion, lowers LDL oxidation and prevents some forms of cancers (Juhel, et al., 2000). It further induces nor-epinephrine linked lipolysis with oolong tea water extract, decreasing lipid absorption and posseses hypoglycaemic and hypoinsuliemia effect in KK-Ay mice (type 2 diabetic and hyper insulinemia mice) after 8 weeks of administration (Miura, 2005). Interestingly, mice fed tea polysaccharides have improved glucose metabolism in use of cold tea extract (Miura, 2005). This suggests that raw green tea (i.e., placed in food) may have added potential to induce weight loss and hypoglycaemia. Green tea influences the increases in adiponectin and LDL particle size in CHF patients (Shimada, et al., 2004). Plasma adiponectin is suppressed during obesity (Haluzik, et al., 2004; Shimada, et al., 2004) and smaller LDL is linked to increased plasma cholesterol, triglycerides and obesity (Suehiro, et al., 1995).

Moreover, a high fat diet supplemented with tea catechins in C57BL/6J mice reduces weight gain, visceral and liver adipose tissue accumulation. These effects are achieved by significant increased liver β oxidation / acyl Co A oxidase (hepatic lipid metabolism) and preventing hyper insulinemia and hyperleptinemia (Murase, et al., 2002). Green tea further stimulates O_2 consumption, BAT and lipid oxidation, hepatic fatty acid oxidation, energy expenditure, adipocyte differentiation and decreasing respiratory quotient in humans (Dulloo, et al., 1999; Wolfram, et al., 2005). Green tea drinkers also enjoy a greater bone density, possibly influenced by flavonoids (Hegarty, et al., 2000).

Chapter 2

General Methods and Materials

2.0 Introduction

Three major studies were undertaken; a sheep model of CHF Cachexia via pace-making implantation, a rat model of weight loss which was induced by 1-Sarcosine-Angiotensin II delivered via osmotic mini-pump, and a mouse diet-induced obesity model with alteration of 2% of diet using different commonly consumed beverages (i.e., cocoa, coffee and green tea). The purpose of these studies was to investigate mechanisms that cause changes in body composition to occur in anorexia/ cachexia and obesity.

The general materials and methods used in all three experiments include the following; general animal details, surgery, analytical methods and experimental procedures. Details of specific procedures, animal species, breed or age, and feeding regimes can be found in the respective chapters dealing with each experiment. All chemicals and reagents used were of an analytical grade. Most reagents were sourced from Sigma-Aldrich and ICN unless otherwise stated.

2.1 Animals (Management)

All animals were checked daily for general health and well-being.

2.1.1 Allocation

Animals were allocated randomly to the different treatments. Whenever necessary, animals were blocked according to their body weight prior to allocation to avoid significant differences in initial body weight.

2.2.1 Diets

Animals were given standard laboratory chow, lucerne/ hay mix or high-fat diet, according to the relevant Chapters 3, 4 and 5-7 respectively. Variations of these diets are specified in the respective chapters.

2.2 Faecal and Urine collection

Within all experiments, individual animals were restrained in their metabolism cages. Details and box specifications can be found in later chapters.

2.3 Blood Collection

Blood samples were collected using heparin (20 I.U. / mL) (in Chapter 4) or EDTA (1mg/mL) (in Chapter 3). To separate plasma from blood, the sample was centrifuged at 5000g for 10 minutes. The blood separated into an upper phase of straw-coloured plasma and a lower red blood cell layer. The plasma was separated off the lower red blood cell pellet using a pipette. Plasma was then stored at -20^{o}C pending analysis.

2.6.1 Feed Consumption

Feed consumption was measured daily by subtracting feed refused from the feed offered on the previous day. In the case of the final experiment involving the development of diet-induced obesity in mice, feed intake was monitored once in 3 – 4 days.

2.6.2 Live-weight

The live weights of rats were measured daily using a balance to a hundredth of a gram and sheep once in each of the three periods using a balance to a tenth of a kilogram as outlined in (Chapter 3) – week 0, week 4, and week 8.

2.6.3 Organ Weights

After the animals were culled, the abdominal cavity was opened and emptied of its viscera and the major organs (i.e., liver, spleen, kidneys, pancreas, lung, heart) were weighed. In the case of the sheep, visceral, omental and peri-renal fat were weighed and also the intestines (small and large) were separated, weighed, emptied and carcass discarded. The mouse and rat carcass' were weighed and then frozen at – 20^{o}C, pending further processing. When processed, the carcasses were minced twice in a commercial meat mincer (die cast 10mm) and a representative sample was obtained and used for chemical carcass analysis.

2.7 Analytical Procedures

2.7.1 Protein Determination

Protein concentration in urine samples were determined according to (Lowry, et al., 1951) and in tissue homogenates according to the Biuret method (Fine, 1935) using bovine serum albumin as a standard. Using the Lowry method, 0.04mL of sample was added to 0.42mL diluent (1% $CuSO.5H_2O$ and 1% NaK tartate.$4H_2O$, 1:1 ratio, then was diluted with 98 volumes of 2% Na_2CO_3 in 0.1M NaOH) and allowed to stand for 10 min. Afterwards, an aliquot of Folin-Ciocalteu's phenol reagent was added and mixed. The reaction was incubated in darkness at room temperature for 20 minutes, and the absorbance recorded at 750 nm.

The Biuret method was used when determining protein concentrates; 20µl of sample was mixed with 180µl of Biuret reagent, incubated in darkness for 30min and read in a micro-titre plate reader (Benchmark BIO-RAD) at 550nm.

2.7.2 Kjeldahl Nitrogen

Kjeldahl nitrogen was determined using the Kjeltec Auto 1035 following manufacturer's instructions. This method is derived from the works of (Kennaway, 1921), and (Stuart, 1936). A portion of dried carcass homogenate sample was weighed (~ 0.5g) and added to a glass digestion tube. 5mL of concentrated H_2SO_4 (ACS reagent 98% purity) was added and the tube was heated to 550^oC on a programmed heating block for 1 hour. A selenium tablet was added to catalyse the reaction. A standard of ammonium sulphate was used to determine accuracy and recovery. The remaining fluid was titrated against HCl using a boric acid indicator. To determine protein content, nitrogen % values were multiplied by 6.25. The co-efficient of variation for the method was 0.44% of the mean.

2.7.3 Hydroxyproline Analysis

The protocol used was an adaptation of Reddy and Enwemeka (1998). A hydroxyproline stock solution of 1mg/mL was made to create a standard curve. The acetate-citrate buffer (pH 6.5) was prepared by dissolving 120g of sodium acetate trihydrate, 46g citric acid, 12mL acetic acid and 34g of NaOH to 1lt of dH_2O. The Chloromine T reagent (0.056M) was prepared by dissolving 1.27g of chloramine T with 20mL of 50% n-propanol and brought to 100mL with acetate-citrate buffer. The 1M Ehrlich's reagent being unstable was made daily using 1.5g of p-dimethylaminobenzaldehyde, dissolved in n-propanol/ perchloric acid (2:1 v/v) and brought to 10mL. To construct the hydroxy-proline standard curve, 10, 7.5, 5, 2.5 and 1.25µl of stock were dispensed in 40, 42.5, 45, 47.5 and 48.75µl of 4M NaOH, reaching a final volume of 50mL respectively. In the case of samples 25µl of sample was mixed with 25µl of 4M NaOH to hydrolyse the samples via heating in an

autoclave at 120^{o}C for 20min. To the hydrolysate, 450µl of chloramine-T was added and oxidation proceeded for 25min. Following this 500µl of Ehrlich's reagent was added and mixed gently before the samples were heated at 65^{o}C for 20min in a water-bath. Absorbency was recorded using a micro-titre plate reader (Benchmark BIO-RAD) at 550nm.

2.7.4 α-Amino Nitrogen Analysis

This method is adapted from the work by (Moore & Stein, 1954). The reagent solution was made by dissolving 5g of ninhydrin with 0.75g hydrindantin in 187.5mL of methyl cellosolve (i.e., 2-Methoxyethanol). The solution was stirred gently to avoid air bubble formation. A volume of 67.5mL of 4N sodium acetate buffer (pH 5.51) was added. The solution was stored in a dark coloured bottle as the reagent is light sensitive.

For each volume of plasma analysed, an equivalent volume of ninhydrin reagent solution was used. A mixture of 50:50 ethanol-water was used as a diluent as necessary. The capped tubes were shaken briefly (>10 sec) and heated in a boiling water bath for 15 minutes. The solutions were allowed to cool and were then lightly shaken and results read in a micro-titre plate reader (Benchmark BIO-RAD) at 570nm.

2.7.5 Glucose

Glucose was measured directly in plasma and urine samples using the glucose oxidase method, adapted from Middleton and Griffiths (1957). The assay reagent consisted of the following; 100mM phosphate buffer (pH 7.0), 0.8U/mL POD, 10U/mL GoD and 1mg/mL ABTS. 5µl of sample was dispensed into 195µl of

glucose oxidase regent in a 200µl microtitre plate well. The reaction was left to proceed for 20min at 37^{o}C and the absorbance recorded at 430nm.

2.7.6 Ash (Bone Mineral Content)

Two sample types were analysed for ash; carcass and feed. All crucibles with lids were initially fired at 550^{o}C for 1hr to remove any residual organic matter. The crucibles were then placed in a desiccator (for 10 minutes) and removed individually for weighing and samples were placed in them and reweighed. The crucible with sample was then fired at 550^{o}C for 5hrs. The crucibles were again placed in a desiccator for 10 mins and removed individually for weighing. The co-variation of the average is 8.4% for the method.

2.7.7 Gross Energy

Gross energy (MJ/kg) of feed and carcasses were measured using a PARR 1261 bomb calorimeter (PARR instrument Co. Illinois U.S.A.). Initially the dried sample was made into a pellet of approximately 0.5g. The pellet was placed in the bomb and combusted. Benzoic acid standards were also combusted to verify reproducibility of data. The co-efficient of variation for the method was 0.19% of the mean.

2.8.1 Isolation of RNA from liver and muscle tissue

Tissues were collected for the purpose of extracting mRNA for analysis of GAPDH (Glyceraldehyde 3-phosphate dehydrogenase), uncoupling 2 and UCP 3. Under anaesthesia, tissue (i.e., liver and skeletal muscle) samples were removed and placed in RNAlater (Ambion, Inc.). The RNAlater was allowed to perfuse the tissue at room temperature for 2 hours, and then the tissue was stored at -70^{o}C pending analysis.

For the extraction of mRNA, ~100mg of tissue was first placed in a manual homogenizer consisting of a glass Teflon® mortar and pestle containing 1mL of Trizol reagent (Life Technologies). The tissue was homogenized until it was dissolved into the liquid reagent. To separate excess protein, fats or polysaccharides, the homogenate was centrifuged at 12,000*g* for 10mins at 4^{o}C. The cleared homogenate solution was transferred to another eppendorph tube and incubated for 5 minutes at ambient laboratory temperatures (approx. 25^{o}C) for the complete dissociation of nucleoprotein complexes, 0.2mL of Chloroform was added per 1mL Trizol reagent used and the eppendorph tube capped and were shaken vigorously for 15 seconds after which the tube and its contents were allowed to incubate at ambient temperatures for a further 2 – 3 minutes. After phase separation, the upper colourless aqueous phase (containing the RNA) was removed and placed in an eppendorph tube. To this, 0.5mL of isopropyl alcohol was added per 1mL of Trizol reagent used and the sample incubated at ambient temperature for 10 minutes and then centrifuged at 12,000*g* at 4^{o}C. The isopropyl alcohol was discarded. The RNA was washed by discarding the supernatant and washing the RNA pellet with 1mL of 75% ethanol. The sample was vortexed and centrifuged at 7,500*g* for 5min at 4^{o}C. Finally, the RNA was redissolved by removing the ethanol and air drying the RNA pellet for 5min. The RNA precipitate was

resuspended in DEPC treated water and stored at -70^{o}C pending further analysis (i.e., qRT-PCR). RNA yield was determined by measuring absorbance at 260nm, and purity (i.e., 1:2:1) by the 230nm:260nm:280nm for the amount of carbohydrate, DNA/RNA, and protein present.

2.8.2 Primer Design

There were four primer pairs designed using published amino acid sequences of UCP 2 and UCP3 species, using the National Centre for Biotechnology information (NCBI) website (www.ncbi.nlm.nih.gov) see Fig 2.1. The primers used in real time PCR were designed and manufactured by Gene Works Pty Ltd (Adelaide, Australia). The primers were stored at –20^{o}C prior to use. The primer sequences are shown on the next page in table 2.1.

Table 2.1 Primers used in RT PCR (sequence, PCR product length, and MW %).

Primer Sequence displayed as Sense first and then anti-sense strand.

Primer Name	Sequence	Length (b.p.)	MW%
Rattus norvegicus UCP3	5’ – TTAAGCCTTCAGCCTTCCATC – 3’		6292
Rattus norvegicus UCP3	3’ – AGAGTCCATCCTGTCCTTCC – 5’	142	6004
Ovis aries UCP3	5’ – AACTGTGGTGAGATGGTGAC – 3’		6237
Ovis aries UCP3	3’ – CCAAAGGCAGAGACAAAGTG – 5’	101	6193
Rattus norvegicus GAPDH	5’ – TTCAACGGCACAGTCAAGG – 3’		5822
Rattus norvegicus GAPDH	3’ – ATACTCAGCACCAGCATCAC – 5’	117	6015
Ovis aries GAPDH	5’ – GTTCCACGGCACAGTCAAG – 3’		5798
Ovis aries GAPDH	3’ – GTACTCAGCACCAGCATCAC – 5’	118	6031

2.8.3 Primer Preparation

Stock solutions of the primers were prepared via re-suspension of the freeze-dried oligonucleotide primers in 100µl of dH_2O. The absorbance (260nm) was recorded using a UV-visible spectrophotometer. The concentration (µM) was estimated using the concentration (µg/mL) by absorbency at 260nm and the molecular weight of the primer. A working solution for the primer was adjusted to 10µM using DEPC treated dH_2O. Both stock and working primer solutions were maintained at –20^{o}C when not in use.

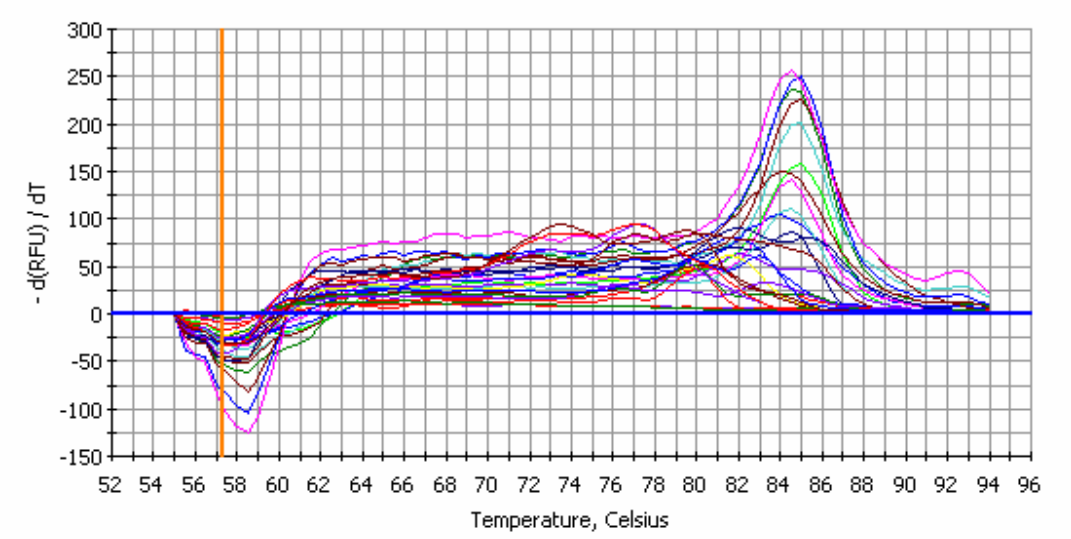
2.8.4 One Step Reverse Transcription of mRNA to cDNA and Quantitative PCR and Melt curve

The iScript One-step RT-PCR kit with SYBR Green was used for reverse transcription and PCR. A total volume of 25µl was pippeted into each well of a PCR plate. It consisted of 12.5µl iScript One-step RT-PCR SYBR Green mix, 1µl of the sense and 1µl of the anti-sense primers, 2µl of ssRNA template, and 7.5µl of nuclease-free H20 and 1µl of Superscript reverse transcriptase were combined and mixed by pippetting. The incubation conditions were as follows; 1 cycle of 42^{o}C for 50min (activation of RTase and conversion of mRNA to cDNA), 1 cycle of 95^{o}C for 1min to de-activate RT-ase and activate Taq DNA polymerase, then 60 cycles of 95 ^{o}C (cDNA denaturation), 60 cycles of 58.3 ^{o}C (primer annealing and cDNA extension), and 60 cycles of 72 ^{o}C (extension termination). The SYBR Green I dye was detected at the extension phase at an excitation maximum at 497nm and emission maxima of 520nm; according to specifications outlined by the manufacturer when using SYBR Green as a fluorophore. The melt curves were conducted using an initial

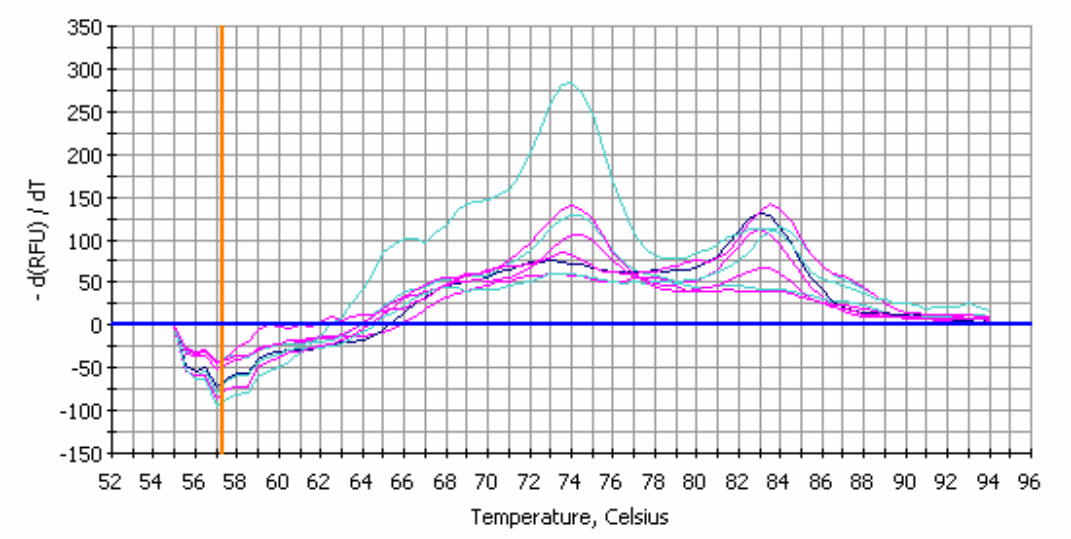
step of 95 ^{o}C (cDNA denaturation) for 1 cycle, and then 80 cycles of 55 ^{o}C (primer annealing) are shown below.

2.8.5 Melt Curve Results

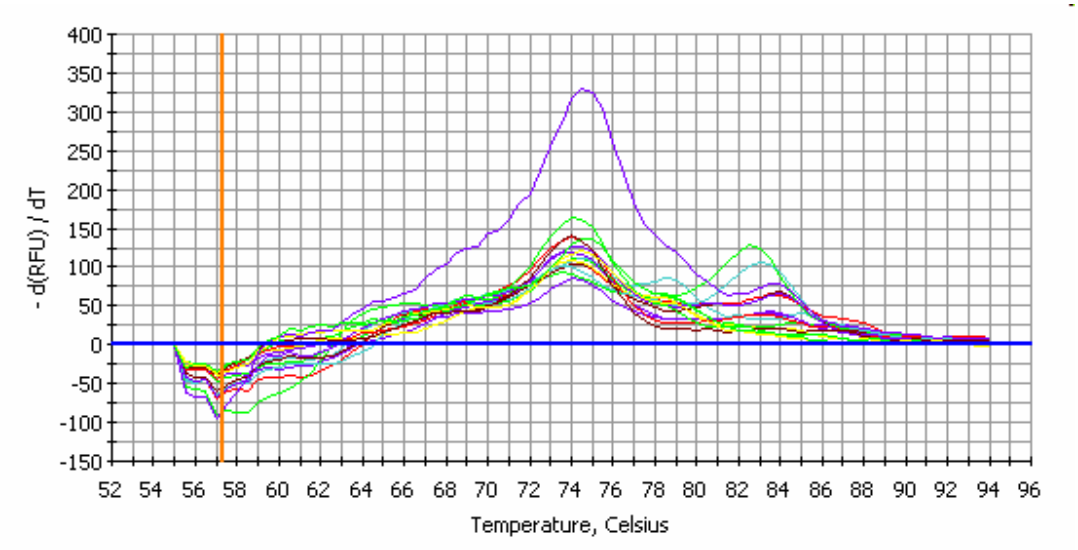
Melt Curve Graph for UCP2 - SYBR-490 – Sheep Liver



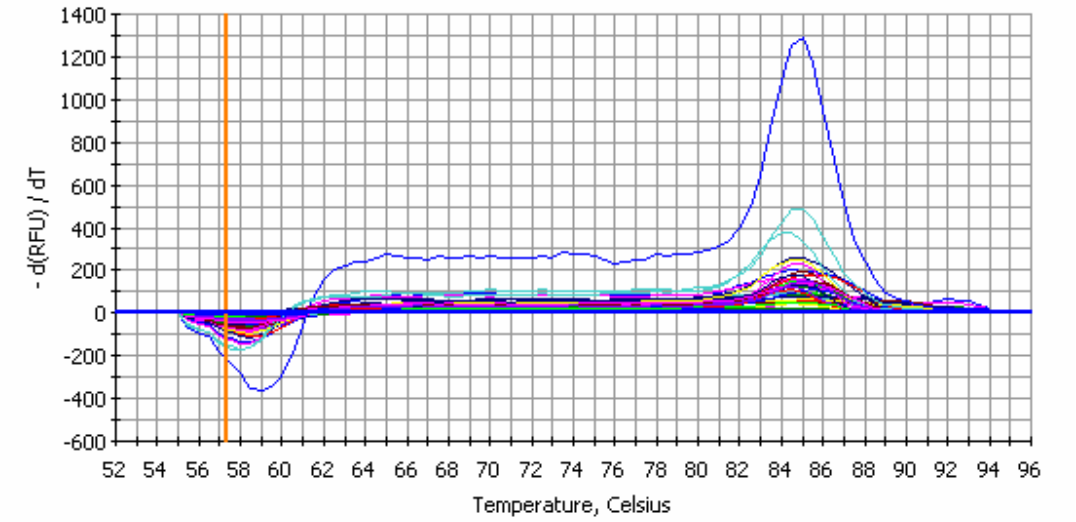
Melt Curve Graph for GAPDH - SYBR-490 – Sheep Liver



Melt Curve Graph for UCP3 - SYBR-490 (Rat Skeletal muscle)



Melt Curve Graph for GAPDH - SYBR-490 – Rat Skeletal Muscle



2.9 Statistical Analysis

All data were analysed for the mean, and standard deviation, standard error, and used either univariate or multivariate analysis. Least squares difference, Turkey's and Bonoforoni tests were conducted using p‹ 0.05. Other forms of analysis are specified in the respective chapters. The statistical analysis was processed using SPSS 12.0.1 for Windows (www.spss.com).

Chapter 3

The Effect of Right Ventricular Pacing (RVP) on Body Weight, and Body Composition Changes in the Sheep

3.1 Introduction

Cachexia is most commonly considered in the context of cancer. It may occur as a consequence of a variety of chronic diseases, with most models focusing on tumour based cancer cachexia in relation to different therapeutic targets (Baracos & Mackenzie, 2006; Emery, 1999; Moe & Armstrong, 1999; Muscaritoli, et al., 2006). The range of animal heart failure models has been mainly employed in rats and mice. Few attempts have been made in sheep. As a result, no suitable large animal model of cardiac cachexia presently exists (Rosenthal & Musaro, 2002). While different methods for inducing cardiac cachexia exist, generally most models stimulate weight loss in adipose tissue and skeletal muscle, decrease energy intake, and increase energy expenditure. Thus, there is a need for the development of a suitable large animal model of cardiac cachexia which closely mimics the human cardiac cachexia prognosis.

A number of animal and human studies show that RVP is implicated in the development of cachexia and that skeletal muscle wasting and elevated energy expenditure are key mechanisms involved in the development of cardiac cachexia. Numerous large animal models (i.e., dog, sheep, and swine) of CHF have been developed. These models imitate composite interactions of cardiac dysfunction, neuro-hormonal processes and “peripheral vascular abnormalities”, which utilize naturally occurring interventions to induce CHF (Power & Tonkin, 1999), which may be used to study cachexia. Various models of RVP exist (Dzemali, et al., 2007; Marcelli, et al., 2007), however the issue of cachexia and, more particularly, skeletal muscle wasting should receive more attention as it is often the

end-point of CHF. Pro-inflammatory cytokines play a central role in the pathogenesis of cardiac cachexia (Sharma, et al., 2001). As a consequence, defining a suitable animal model for the study of possible treatment methods is an important goal for the study of the pathophysiology of cachexia and use of a model to study possible treatment methods. Another factor effecting the investigation of cachexia diagnosis and prognosis is a lack of general definition. (Coletti, 2008) described that the prevalence of cachexia is underestimated, misdiagnoses and a range of conflicting data are reported. A common disadvantage of many animal models of cachexia is that very few reproduce clinical settings (e.g. poly-medicated patients) and many lack human inflammatory responses (Coletti, 2008).

A large proportion of CHF and cardiac cachexia studies have employed the rat to establish a model (Delafontaine & Brink, 2000; Schulze, et al., 2003b). Due to the similartity of sheep to humans in cardiac profile, sheep were chosen for this model as (Cissik, et al., 1991) observed many similarities with the human heart (e.g., heart rate, cardiac output, and arterial, pulmonary artery etc.) and the ovine hearts are most similar to human in adult size (Power & Tonkin, 1999). There has been development of an ovine model of left ventricle dysfunction to study the pathogenesis of cardiac remodelling (Hasnat, et al., 2003). Given the close similarities between the human and sheep heart it is suggested that the two have matching cardiac physiological parameters which make sheep a suitable animal for the study of cardiac cachexia induced by RVP.

This study was initiated upon the preliminary observations by Dr. John Power (*Pers. Comm.*)(Baker Medical institute, Melbourne, Australia), who observed loss of thoratic skeletal muscle in sheep following mechanical RVP. The present study explores the use of a RVP system to characterise the physiological responses to different rates of pacing in the adult sheep to induce cardiac cachexia development.. To fully characterise the effects of RVP, a wide range of plasma physiological markers, energy kinetics and gene expression were made in addition to body weight and food intake observations. Skeletal muscle UCP 2 mRNA (Uncoupling Protein 2) was measured to explain any body

weight or composition changes that may be linked to associated oxidative stress and elevated energy expenditure (e.g., addressing proton leak and mitochondrial inefficiency) (Boss, et al., 2000; Pecqueur, et al., 2001). The present study tested the hypothesis that a steady increase in RVP (as described in Chapter 3.2.7) for at least 8 weeks would induce CHF Cachexia in wethers.

3.2 Materials and Methods

3.2.1 Animals

All sheep (n = 13) used in the experiments were adult (Merino x Border Leicester) wethers. In the pair-fed group there were n = 6. In the RVP group there were n = 7 sheep. The animals were selected as having similar body weight and age. The weight ranges for the animals were between 45 – 55kg. The sheep were sourced from VIAS (Victorian Institute of Animal Science), Werribee. Prior to experimentation, animal ethics committee approval was granted (VIAS, Werribee).

3.2.2 Housing

The wethers were accustomed to feed and cage constraint for at least 1 week before commencement of the baseline metabolic study. Throughout all studies, sheep were maintained in metabolic cages (1.2m x 1.6m x 1.0m respectively). The sheep were housed at temperate temperatures in an enclosed shed.

3.2.3 Diets

The ewes were offered an oaten chaff/lucerne chaff mixture (1:1) (Freemans, Fremantle WA). The sheep were offered 1.8kg/d which is above maintenance for a 40kg sheep for both RVP (Right Ventricular Paced) sheep (n = 6) and pair fed controls (n = 7, with n = 1 withdrawn after week 1 due to illness). The sheep were fed twice daily with *Ad libitum* access to water.

3.2.4 Faecal and urine collection

At the commencement of the study (baseline), week 4 and week 8 periods, a metabolic study was performed over 7 days. Bags were placed under the metabolism cages (perforated in one corner to drain any contaminant urine) and faeces were collected into bags and weighed. A 500g sub-sample of faeces per sheep per period was collected and 1mL/kg 37% formaldehyde (w/v) was added and a homogeneous sub-sample frozen at -20^{o}C was collected for further analysis. Urine was collected in buckets under the metabolism cages and then 5mL of concentrated sulphuric acid was added per litre of urine collected. A daily 200mL sub-sample was collected and frozen at -20^{o}C. Other parameters recorded were; live body weight (kg), feed consumed (kg) and water consumed (L). The faeces were air-dried in a fan forced oven at 60ºC and dry weight recorded. The faeces were ground into a fine powder. The energy content was determined using a bomb calorimeter (see Chapter 2.7.1.1). Also the nitrogen content of feed, faeces and urine were determined via the Kjeldahl method (refer to Chapter 2.7.2).

3.2.5 Nitrogen balance

The nitrogen balance was determined by analysing the nitrogen concentration of the feed, faeces and urine, conducted using the Kjeldhal apparatus (see Chapter 2.7.2).

3.2.6 Body weight

The live weight of the wethers was recorded to the nearest kilogram at baseline, week 4 and week 8, using a measuring scale.

3.2.7 Surgery and Pacemaker implantation

After baseline week 0, initial surgical preparation included the installation of a RV pacing lead and pacemaker. The surgical operations were performed by Dr. John Power and Dr. Melissa Byrne. The wethers were paced at 190 bpm. pacing threshold for 3 weeks. Next, the RV was paced at 210 bpm. and four-fold pacing threshold for another five weeks.

3.2.8 Catheters

The wethers were surgically prepared with indwelling catheters 8cm in both jugular veins; carotid artery and iliac vein (12-14cm). The size of the catheters were (9mm id and 11mm ed). On every second day of the study, the catheters (carotid, left and right jugular (contra lateral) and iliac veins) were flushed and kept patent. They were flushed in the following manner; the catheter line outside the animal's body was clamped off with cushioned ended artery forceps. The catheter was flushed once with sterile saline (~2mL), and withdrawn to clear the catheter with fresh blood. Next the catheter was flushed again with sterile saline (3mL) and filled with 2mL of EDTA (3g/L).

3.2.9 Blood collection

Blood samples were collected from the sheep at baseline, week 4 and week 8, when performing the palmitate and tyrosine infusions. The time intervals for blood collection were 0, 160, 170, 180, 240, 270 and 300min following the commencement of infusion. Blood samples (9mL) were obtained from three vessels; carotid artery, jugular vein and iliac vein for separation of plasma. An additional sample at time 0 was collected to prepare serum. The blood was allowed to clot at room temperature for 4 hours then kept at 4^{o}C overnight before separating serum.

The serum was removed and stored at –20^{o}C pending further analysis. The blood for plasma was collected into vacutainer containing EDTA and was stored on ice immediately following collection. Within 1hr the blood was centrifuged at 3000*g* for 15min at 4^{o}C and plasma yielded. The plasma was removed and stored at –20^{o}C pending further analysis.

3.2.10 DEXA (Dual X-Ray Absorptiometry)

In preparation for the DEXA analysis, sheep were shorn one week prior to measurements being taken. The sheep were anaesthetised with (0.8L/min of 2-5% halothane) and the animal was artificially respired. The animal was scanned with the DEXA. Bone Mineral Area (BMA) (g/cm^{2}) (using lumbar vertebrae), BMC (bone mineral content), lean tissue and fat mass were determined for the whole body, leg and lumbar vertebrae. The DEXA machine was calibrated using the chemical composition of sheep carcass.

3.2.11 Palmitate Infusate - Preparation and infusion

The infusate was prepared one day prior to infusion and was kept at 4^{o}C pending use. The [9, 10 -^{3}H] Palmitate (72μCi) (ICN) was added to 3 mg of cold (non-radioactive - carrier) palmitate and 10 μl of 4M KOH. After the mixture solubilized it was dried under a gentle stream of nitrogen. The K^{+} soap was dissolved in 10ml of warm saline (60^{0}C), and was incubated for 30 min at 60^{0}C. After the solution had dissolved, it was filtered through a 0.22 μm filter (Millipore) into 15ml of sheep plasma freshly prepared from the animal. Warm sterile saline was added to dilute the final volume to 50ml.

The palmitate entry rate was determined by continuous infusion technique. [9, 10 -^{3}H]-labelled palmitate was infused at a rate of 20.53nCi per min for approximately 3 h using a syringe pump at 0.15ml infusate/min. All glassware was autoclaved and aseptic conditions maintained at all times.

3.2.12 NEFA Extraction and analysis for palmitic acid

To 1mL of plasma on ice, 100μl (1nCi) of internal standard ([1-C^{14}] Palmitic acid) was added to determine recovery of extracted NEFA. After mixing plasma gently by inversion, 6mL of Dole's reagent (isopropanol: heptane: 2N H_2SO_4, 40:10:1, (v/v/v)) was added and vortexed. A further 2mL of heptane was added and mixed, then 1mL of distilled water was added and vortexed. The upper phase was transferred to a clean tube and 2mL of heptane was added and the extraction repeated. The lower phase containing non-lipid substances was added to a scintillation vial and counted. The pooled heptane fractions were extracted with 3mL (3% K_2CO_3: ethanol: iso-amyl alcohol (40:59.6:0.4, v/v/v)) and the upper heptane phase was discarded. The lower phase containing NEFA as K^{+} soaps was washed twice with 3mL of heptane. The NEFA is extracted back into the heptane by adding 3mL of heptane to the aqueous alcohol solution and acidifying with 3 drops of 27N H_2SO_4. After mixing and allowing phase separation, the heptane layer was transferred to another extraction tube. The alcoholic aqueous phase was extracted with a further 2mL heptane. The pooled heptane layers were evaporated to dryness under a gentle stream of nitrogen. The NEFA's were converted to methyl esters by refluxing

in 2mL 5% H_2SO_4 in methanol (60°C for 2 hr). 2mL of heptane was added and mixed followed by 1mL of 4% NaCl. After mixing, the methyl esters of Palmitic acid were transferred to a scintillation vial. The lower phase was washed with another 2mL heptane and the pooled heptane fractions dried under a stream of nitrogen. The FA methyl esters were reconstituted in 25µl of heptane. 0.5ul of this sample was reserved for GLC (gas liquid chromatography).

3.2.13.1 Non-esterified free fatty acids concentration

The determination of non-esterified free fatty acid concentration in plasma was conducted using the NEFA C (ACS-ACOD Method) commercially produced by Wako, Osaka, JAPAN. Following the manufacturer's guidelines, 10µl of sample was added to 65µ1 of colour reagent A in a microtitre-plate well and mixed via pippetting. The mixture was allowed to incubate at 37^{o}C for 10 minutes. After incubation was complete, 125µl of colour reagent B was added and again the mixture was allowed to incubate at 37^{o}C for 10 minutes. The optical density was measured at 550nm, a standard curve drawn and NEFA concentration determined, using palmitic acid as a standard.

3.2.14 Organ weights

At the completion of the experiments, sheep were killed by opening the thorax and excising the heart whilst the animal was under anesthesia. The organs were removed from the culled animal and dried of excess blood with paper toweling. The organs were weighed and their mass recorded for heart, spleen, pancreas, kidney, lungs, liver and gall bladder. The rumen and intestine were initially weighed full of digesta and then emptied of their contents and re-weighed; determining digesta weight. In addition the visceral adipose tissue (omental, peri-renal and mesenteric) was weighed.

3.2.15 Palmitate turnover

The specific radioactivity of palmitate was calculated using the following equation;

$$\frac{(2200/\ ^{14}C\ cpm) \times\ ^{3}H\ cpm}{\text{nmoles of palmitate in 1mL palmitate}}$$

Palmitate R, (pmol/kg/min) = [U-"C] palmitate infusion rate (pmol/kg/min)/ plasma palmitate APE.

3.2.16 Glucose

Glucose concentration of plasma was determined using the glucose oxidase method (see Chapter 2.7.5).

3.2.17 Alpha-amino nitrogen

α-amino nitrogen concentration of plasma was determined as outlined in Chapter 2.7.4.

3.2.18 Uncoupling Protein Expression

Please refer to Chapter 2.8.5

3.2.19 Statistical Analysis

The body weight was analyzed using one-way repeated measures ANOVA. Data for organ weights, plasma α-amino nitrogen, and glucose were analyzed using a one-way ANOVA. The DEXA data, feed intake, creatinine, nitrogen balance, NEFA and palmitate turnover were analyzed using multivariate analysis. ANOVA and LSD (least squares difference) were analyzed at $p<0.05$ and $p<0.01$. These analyses were conducted using SPSS Version 12.

3.2.20 Experimental time course and procedure

Baseline	**Week 1 - 3**	**Week 4 – 7**	**Week 8**
Sheep acclimatized to feed and housing	Surgery	Nitrogen balance	Nitrogen balance
Nitrogen balance	Pacemaker implantation	DEXA & Body Weight	DEXA & Body Weight
DEXA & Body Weight	Pacing at 190 bpm	Pacing at 210 bpm	Palmitate turnover study
Catheter implantation	Palmitate turnover study		Organ weights recorded
Palmitate turnover study			Samples collected for plasma (NEFA, glucose, amino nitrogen)
			UCP2mRNA analysis

3.3 Results

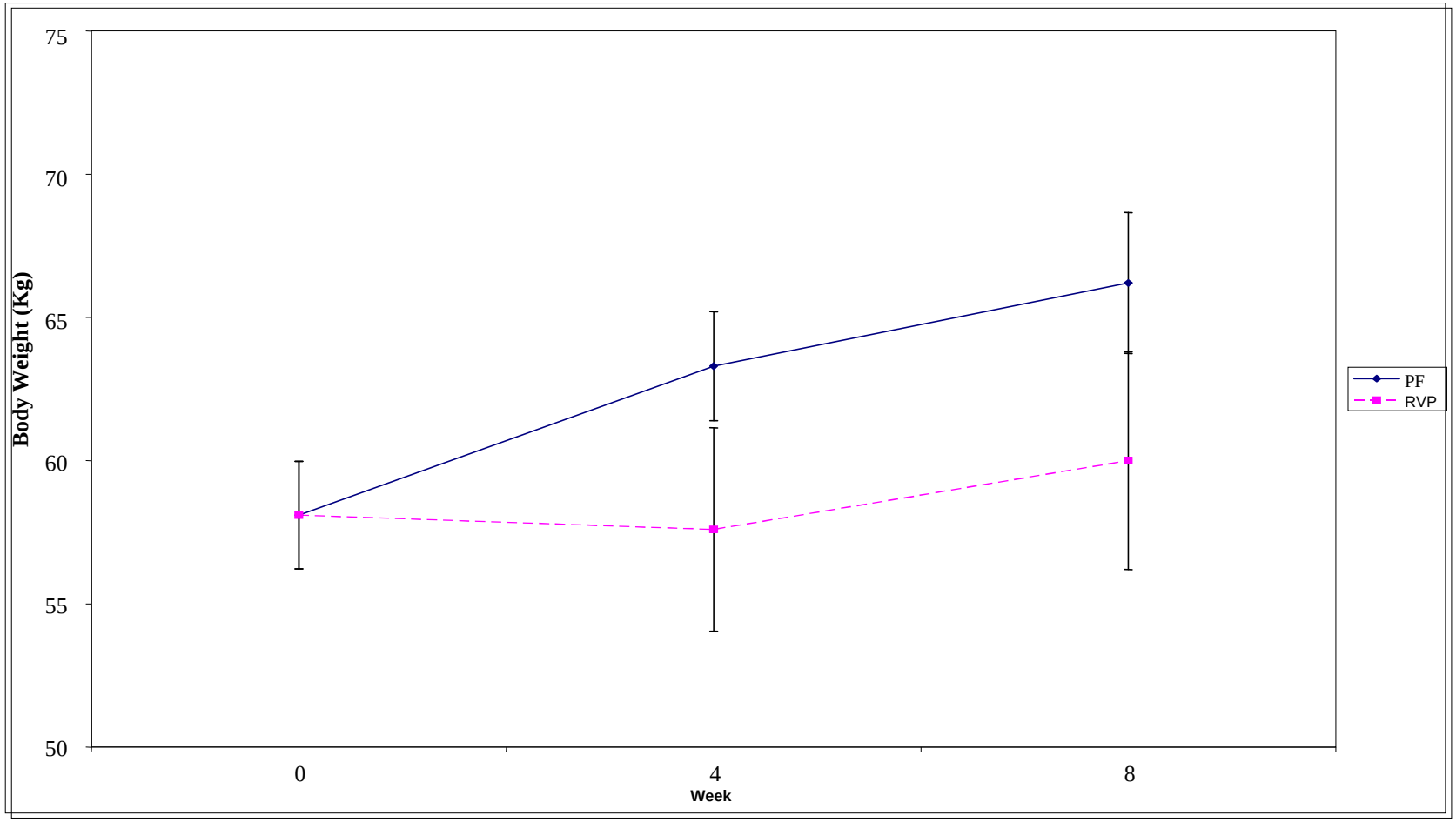


Figure 3.1 Progressive live body weight over an 8 week period for PF (Pair fed) and RVP (Right ventricular paced). Data displayed as mean ± standard error.

3.3.1 Body Weight

When viewing the progressive body weight over the 8 weeks (Figure 3.1) for both groups, there was no significant difference between treatments.

3.3.2 Organ Weights

From Tables 3.1 - 3.2, and Figure 3.2 - 3.4, with comparision with the pair fed group, the RVP group displayed a decrease ($p<0.05$) in pancreas (39%) and an increase ($p<0.05$) in visceral adipose tissue weight (23.7%) on a per kg basis. The heart (29%), pancreas (33%), and visceral adipose tissue (23.7%) (in the RVP group) as a percentage of live weight basis were increased ($p<0.05$) in comparison to the pair-fed control.

Table 3.1 Organ Weight and body weight (kg) for RV paced and pair-fed control sheep at 8 weeks

Organ (kg)	PF (kg)	RVP (kg)
Heart	0.52±0.06 (6)	0.65±0.04 (7)*
Spleen	0.212±0.01 (4)	0.298±0.05 (6)
Kidney	0.225±0.01 (6)	0.296±0.04 (7)
Lung	1.1±0.08 (6)	1.27±0.12 (7)
Liver	1.37±0.04 (6)	1.56±0.11 (6)
Pancreas	0.064±3.9 (5)	0.038±9.6 (7)*
S. Intestine (full)	0.88±0.02 (4)	0.75±0.11 (3)
S. Intestine (empty)	0.67±0.99 (4)	0.55±0.11 (3)
L. Intestine (full)	5.14±0.39 (4)	5.98±0.26 (3)
L. Intestine (empty)	1.84±0.12 (4)	1.85±0.17 (3)
Rumen (full)	9.8±0.58 (4)	10.96±0.4 (5)
Rumen (empty)	3.1±0.1 (4)	3.4±0.2 (5)
Visceral Fat	3.02±0.4 (4)	4.08±0.3 (4)*
Live Body Weight	66.2±2.0 (6)	60.0±3.8 (7)

The results are displayed a mean± standard error (number of observations). *Signifies a significant difference between treatments $p < 0.05$.

S. = Small and L. = Large

Table 3.2 Organ Weights as a proportion of live body weight (kg); PF Controls and RVP sheep at 8 weeks

Organ (kg)	PF controls	RVP
Heart	0.79±0.07 (6)	1.08±0.05 (7)*
Spleen	0.32±0.03 (4)	0.5±0.1 (6)
Kidney	0.34±0.02 (6)	0.53±0.01 (7)
Lung	1.68±0.16 (6)	2.24±0.34 (7)
Liver	2.07±0.03 (6)	2.72±0.34 (7)
Pancreas	0.1±0.008 (5)	0.059±0.012 (7)*
S. Intestine (full)	1.3±0.22 (4)	1.11±0.21 (3)
S. Intestine (empty)	1.0±0.13 (4)	0.81±0.21 (3)
L. Intestine (full)	7.7±0.68 (4)	8.67±0.19 (3)
L. Intestine (empty)	2.77±0.24 (4)	2.67±0.18 (3)
Rumen (full)	14.61±0.7 (4)	17.7±1.4 (5)
Rumen (empty)	4.46±0.24 (4)	5.5±0.64 (5)
Visceral Fat	4.49±0.4 (4)	5.78±0.3 (4)*

The results are displayed a mean± standard error (number of observations).
*Signifies a significant difference between treatments $p < 0.05$.
S. = Small and L. = Large

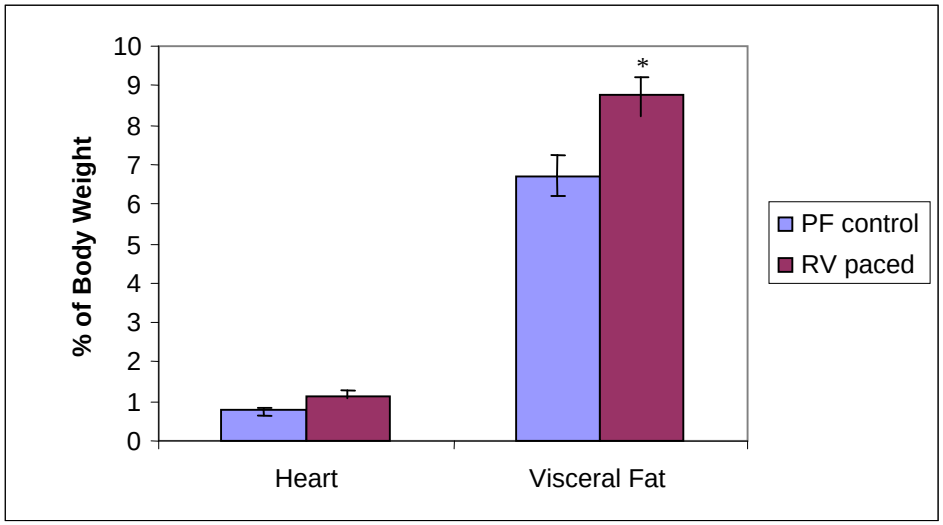


Figure 3.2 Heart and Visceral Fat (as a percentage of body weight). Data are expressed as mean±standard error. *Signifies a significant difference between treatments p <0.05.

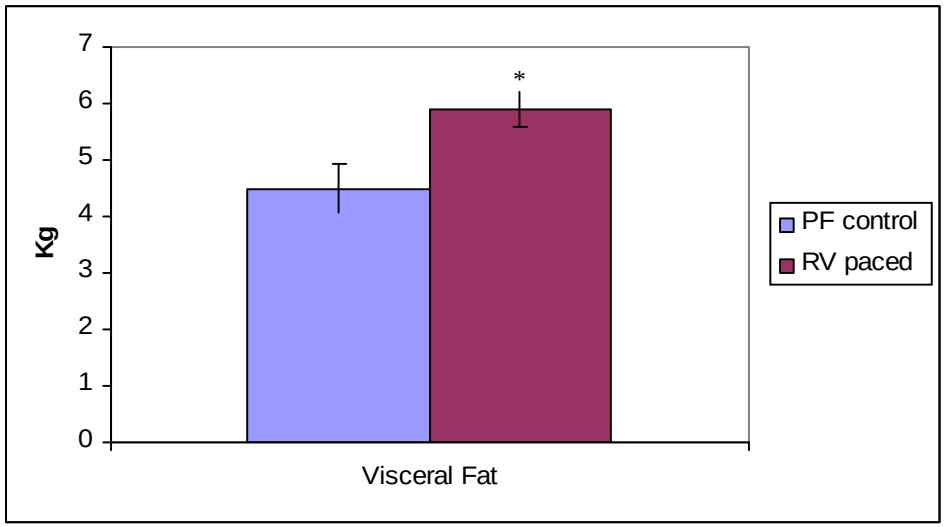


Figure 3.3 Visceral fat (Kg). Data are expressed as mean±standard error. *Signifies a significant difference between treatments p <0.05.

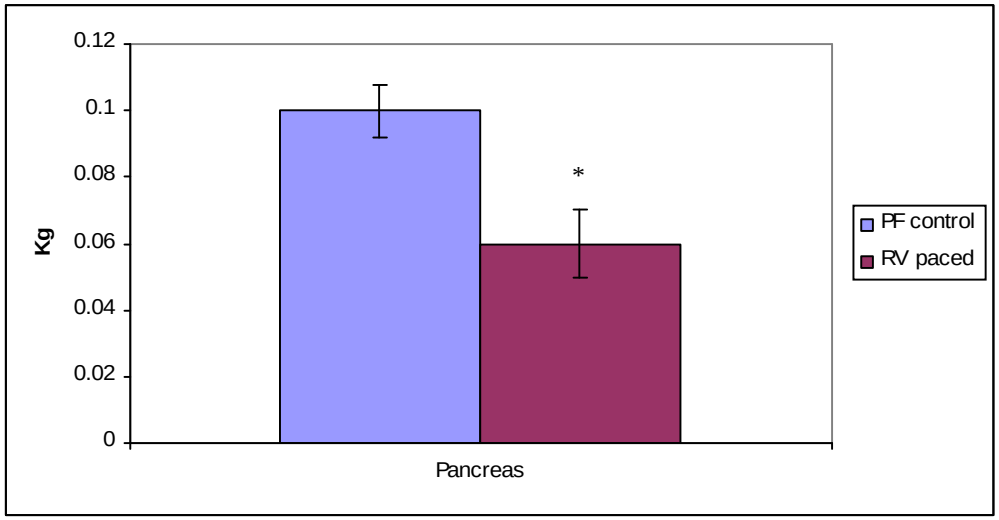


Figure 3.4 Pancreas (per Kg). Data are expressed as mean± standard error. *Signifies a significant difference between treatments p <0.05.

Table 3.3 DEXA – Body Composition Data at initial (Week 0), week 4 and week 8 (terminal) for both PF and RVP

Tissue	initial (Week 0)	PF (Week 4)	RVP (Week 4)	PF (Week 8)	RVP (Week 8)
BMA (cm^2)	1.35±0.03 (13)	1.4±0.08 (6)+	1.3±0.08 (6)	1.5±0.04 (6) +	1.4±0.1 (6)
Ash (kg)	1.65±0.03 (13)	1.7±0.1 (6)	1.5±0.1 (6)	1.8±0.3 (6)	1.9±0.2 (6)
Adj. Fat (kg)	8.73±1.6 (13)	9.7±1.7 (6)	9.2±1.8 (6)	7.9±2.1 (6)	9.4±2.8 (6)
Adj. Lean (kg)	38.6±3.7 (13)	43.9±3.5 (6)	36.5±4.4 (6)	44.4±4.9 (6)	38.4±3.6 (6)
Adj. Total (kg)	54.1±3.3 (13)	60.7±2.4 (6) +	51.5±6.8 (6)	61.1±6.7 (6)	52.8±6.3 (6)
BMA (kg) leg	0.21±0.01 (13)	0.21±0.01 (6)	0.19±0.01 (6)*	0.19±0.02 (6)	0.2±0.01 (6)*
BMC (kg) leg	0.21±0.01 (13)	0.22±0.02 (6)	0.18±0.02 (6)*	0.24±0.07 (6)	0.2±0.02 (6)*
Fat (kg) leg	0.93±0.18 (13)	0.92±0.15 (6)	0.98±0.19 (6)	0.58±0.03 (6)	0.82±0.46 (6)
Lean (kg) leg	5.9±0.5 (13)	6.4±0.9 (6)	5±0.6 (6)+	6.4±0.7 (6)	5±0.3 (6)+
Total (kg) leg	7.0±0.45 (13)	7.6±0.8 (6)	6.2±0.7 (6)+	7.4±0.7 (6)	5.9±0.3 (6)+

The results are displayed a mean± standard error (number of observations).
The Fat, Lean and Total are all adjusted (Adj.) values, adjusted with chemical composition of carcass. Hunter T.E. (2000).
*Signifies a significant difference between treatments at a level of $p \leq 0.05$
+Signifies there is an interweek significant difference in the same treatment.

At the end of the study (refer to Table 3.2), the RVP group displayed increased ($p<0.05$) visceral fat. In all weeks (refer to Table 3.3), the pair fed group displayed a significant inter-weekly increase in BMA. Further, in week 4 the RVP group displayed decreased ($p<0.05$) BMA, and lower BMC ($p<0.05$) and lower lean leg content in the leg (week 8). Lastly, the RVP group showed a decreasing trend in total leg mass (kg).

From Table 3.4 there is no difference in feed intake or nitrogen balance between the two groups. However, there is an increased ($p<0.05$) inter-weekly difference between week 0 and week 4 for feed intake for both the pair-fed and RVP group.

Table 3.4 Feed intake (g/d) and Nitrogen balance (g).

	Week 0		Week 4		Week 8	
	PF	RVP	PF	RVP	PF	RVP
Feed intake g/d	1474±69 (13)	1435±70 (13)	1651±75 (6)+	1619±70 (6) +	1660±75 (6)	1633±69 (6)
Nitrogen Balance (g/7d)	-7±2.6 (13)	-6±2.6 (13)	-11±2.8 (6)	-8±2.6 (6)	-10±2.8 (6)	-9±2.6 (6)

The results are displayed a mean± standard error (number of observations).
[+]Signifies there is an inter-week significant difference in the same treatment.

Table 3.5 Arterial Venous Difference (mM) for α-amino nitrogen at week 8.

	Pair Fed	RVP
Arterial Venous Difference	0.23±0.037 (6)	-0.75±0.036 (6)*

The results are displayed a mean± standard error (number of observations).
*Signifies a significant difference between treatments at a level of $p \leq 0.05$

Table 3.6 Glucose concentration in the carotid artery and the iliac vein at week 8.

Metabolite	Pair-Fed	RVP
Carotid Artery (Glucose)	2.9±0.184 (6)	3.2±.0.16 (6)
Iliac Vein (Glucose)	3.1±0.36 (6)	2.9±0.29 (6)

The results are displayed a mean± standard error (number of observations).

Table 3.7 Turnover Data (Palmitate) (µmol/min/kg) and plasma NEFA (Non-esterified fatty acid) concentration.

	Week 0 Pair-Fed	Week 4 Pair-Fed	RVP	Week 8 Pair-Fed	RVP
Palmitate Turnover	2.46±0.5	3.6±0.5	3.62±0.4	2.2±0.5	1.9±0.5
NEFA (mM)	0.305±0.035	0.3±0.04	0.32±0.03	0.283±0.04	0.31±0.04
Energy Expenditure per period (MJ)	n/a	48.9±4.3	40.3±2.41	36±7.26	54.5±9.66*

The results are displayed a mean± standard error (number of observations).
*Signifies a significant difference between treatments at a level of $p \leq 0.05$

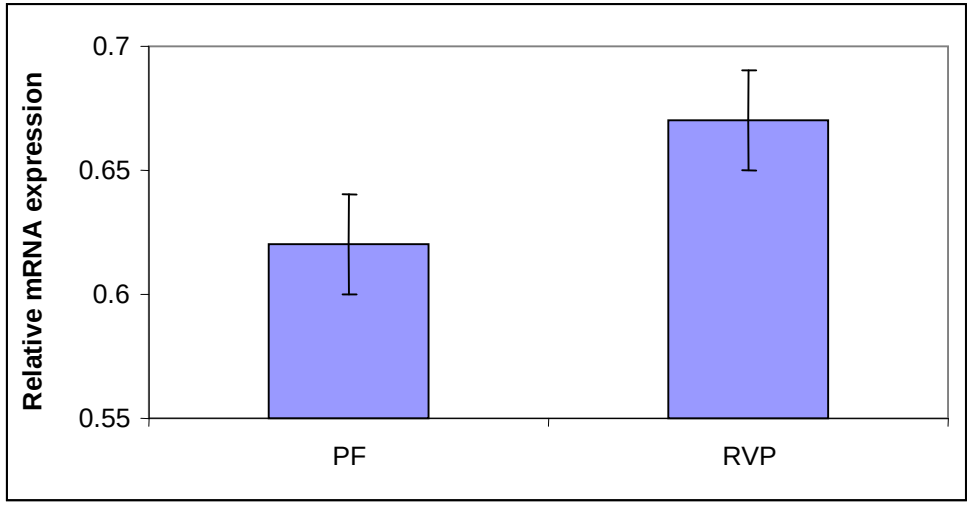


Figure 3.5 Comparative gene expression of uncoupling protein 3 in skeletal muscle (SKM) in sheep at week 8 in PF and RVP. The results are displayed a mean± standard error (number of observations). Units are arbitrary units (Gene of interest/ house keeping gene; GAPDH).

The RVP sheep displayed a significant negative loss of α - amino nitrogen (Figure 3.5) from the hind-limb via the iliac vein in comparison to the pair fed controls. Further, glucose concentrations are not different at week 8 (Fig. 3.5 and 3.6).

From table 3.7 it can be seen that there is no significant difference in palmitate turnover or plasma NEFA concentration (mM). However during week 8, the RVP group display elevated ($p<0.05$) energy expenditure. There was no change in UCP3 gene expression in skeletal muscle between groups (Figure 3.5).

3.4 Discussion

It was hypothesized that right ventricular pacing is a useful method to achieve a suitable large animal model to study cardiac cachexia. This aim was successfully achieved, as the present study demonstrates that there was wasting of skeletal muscle and decreased body weight (non-significant) following RVP in sheep. There is a significant decrease in leg lean mass and BMC in the RVP group yet an increase in visceral fat to that seen in pair-fed control sheep. In all weeks, the PF group displayed a significant inter-weekly increase in BMA. There was no difference in feed intake, nitrogen balance, plasma NEFA, palmitate turnover between the two groups, yet a significant negative loss of α - amino nitrogen from the hind-limb in the RVP gorup. However during week 8, the RVP group display elevated energy expenditure yet no change in UCP3 gene expression in skeletal muscle between groups.

Increased visceral fat mass and decreased lean tissue of the arms and legs are hallmarks of CHF cachexia in humans. These findings suggests that a change in the activity of lipogenic hormones and muscle catabolic factors is present, thus altering fat metabolism. Mechanistically, research in malnourished humans with cachexia, shows that elevations of pro-inflammatory cytokines TNF-α, IL-1β and IL-6 (Cederholm, et al., 1997) promote cachexia and alters fat metabolism (Tocco-Bradley, et al., 1987).

In agreement with the primary hypothesis, the present study has found that at week 8 there was an increase in body weight (66kg versus 60kg) (figure 3.1), however, due to the large standard error, a statistical difference was not observed. A consideration is the number of animals in the study. With a greater number of subjects, a longer observation period (e.g., 12 – 15 weeks) this standard error would be reduced. In addition, due to the increased visceral fat pad, the animals externally appeared of comparable weight, but had an altered body composition. Heart failure patients have been noted to develop anemia and lower BMI (Colin-Ramirez, et al., 2006), and cardiac hypertrophy (Korstjens, et

al., 2002). A proposed mechanism causing this lean leg atrophy, could be due to anemia, as there would be less oxygenated blood supply to skeletal muscle and thus reduced bundle size. Conversely, chronic and rapid ventricular pacing has been observed to produce congestive heart failure and increased body weight in dogs (Howard, et al., 1988). The animals in this study were offered a standard amount of feed each day (i.e., pair fed to RVP sheep), however in other studies animals ate *ad libitum*, thus explaining the differences in findings (i.e., if the control animals were able to eat more they may have gained more weight, thus a statistically significant heavier body weight and possibly more profound changes in body composition as well). The body weight results support an earlier study which suggested that visceral and subcutaneous abdominal adipose tissue were more significant predictors of the development of CHF than overall total body obesity (Nicklas, et al., 2006). The significance of these findings demonstrates that neither skeletal muscle growth, not body weight,was dependant on feed intake, and other catabolic factors need examination to explain involuntary weight loss during RVP.

It was also predicted that during and after RVP, the heart would undergo enlargement. Cardiac hypertrophy was a key result of RVP in sheep in the present study. In a similar study examining CHF; heart and lung weights as a proportion of live body weight were found to be greater in mass (Evans, et al., 2003), particularly the heart and kidney mass. In the present study, the heart mass was increased as a percentage of body weight in accordance with (Verduyn, et al., 2001) who observed left ventricle and right ventricle hypertrophy after 19 days of atrio-ventricular block (CAVB) in dogs, as did Levett, et al., (1994), when studying atrial hypertrophy in dogs. Moreover, (Chow, et al., 1990) induced right ventricular pacing in pigs and found significantly greater heart weight-to-total body weight ratio after 7 days. Cardiac hypertrophy was greater in the RVP group, which was linked to significant up-regulation of inducible nitric oxide synthase, which inhibits mitochondrial complex IV, resulting in a bioenergetic defect and a possible indicator of CHF (Brookes, et al., 2001). Interestingly, Azelnidipine suppresses cardiac hypertrophy via down regulation of superoxide production, thus reducing cardiac oxidative

stress, inflammation and fibrosis (Yamamoto, et al., 2006). This may prove to be a suitable form of treatment for cardiac hypertrophy, rectifying not just cardiac, but tissue oxidative damage and stress. Changes in organ weights, particularily the heart, are highly important as the heart regulates cardiac output and blood pressure. Alterations in these two parameters may further worsen prognosis of cachexia via imbalance of pro-inflammatory hormones.

Loss of lean tissue and BMC in RVP sheep were clear signs of cachexia. Lean tissue and BMC loss are well-established effects of cardiac cachexia (Berry, et al., 2000; Norrelund, et al., 2006). Additionally, Anker, et al., (1999) found that cachectic CHF patients had significant wasting of adipose tissue, in addition to lean tissue and bone mineral tissue in arms and leg region. In the present study, there was no adipose tissue-related wasting, and conversely there was visceral adipogenesis. A proposed mechanism of decreased BMC in this study, reduced bone mass, is a common factor in heart failure, and is induced via lower serum vitamin D and secondary hyperparathyroidism (Berry & Clark, 2000). Decreasing bone density will contribute to the increased fatigue experienced by patients with CHF cachexia.

Despite the changes in body composition, it may have been interesting to have offered the sheep an *Ad libitum* feeding program. Thus, it could be suggested the levels of the appetite regulating hormones NPY and leptin were not suppressed or elevated, repsectively during the 8 weeks of RVP. However, without direct measurement of these compounds, conclusions relating to the action of these hormones would be speculative. The mechanism by which energy intake is controlled in cardiac cachexia is thought to be through ghrelin. Ghrelin may prove to be a useful method of treatment to restore BMI through improved appetite (Akamizu, et al., 2007; Coletti, et. al. 2008), as does clenbuterol (i.e., promotes weight gain via lean mass) (George, et al., 2006). Some researchers (Palmieri, et al., 2008) have found that heart failure in elderly patients with a left ventricle ejection fraction < 45%, had lower body mass index, adipose mass, fat-free mass, and lower 24-hour calorie

intake than participants with normal ejection fraction (>55%). It could be suggested that the sheep in this study had unaltered plasma ghrelin levels and higher ejection fraction (i.e., ≥55%) as body composition was not greatly disturbed during pacing. Also there was negative nitrogen balance in both groups with no difference between groups.

Consistent with these suggestions, the decrease ($p<0.05$) in leg lean tissue (kg) in the RVP sheep in week 4 and week 8, suggests that there may be alterations in oxygen consumption and energy expenditure. Alterations in body composition may alter absolute peak oxygen consumption and carbon dioxide production during CHF as observed by (Cicoira, et al., 2001), and is agreement, with findings by (Toth & Matthews, 2006), and (Piepoli, et al., 2006), who observed significant reduction in muscle mass in cachectic CHF patients. Notably, elevated REE in CHF patients is linked to increased protein catabolism as measured by leucine appearance rate (Toth & Matthews, 2006). When considering these factors, it may be possible to propose that the efflux of leg amino acids seen via measurement of differences in α-amino acids between the carotid artery and iliac vein is linked to elevated oxygen consumption.

There are studies which suggest that gastrointestinal impairment during CHF leads to nutritional deficiencies of vitamins and minerals (i.e., Zinc, Selenium) via reduction in dietary intake, leading to decreased absorption due to gastrointestinal oedema (Cohen, et al. 2006; Witte, et. al. 2006). In agreement, the present study shows that there was a larger increase in the amount of intestinal digesta seen in the RVP group, which may indicate mild gastrointestinal system impairment in CHF, causing malnutrition (Krack, et al., 2005). Alterations in digestive absorption may have altered protein metabolism and thus influenced loss of lean tissue.

RVP did not cause a significant difference in nitrogen balance between groups, but in both the RVP and pair fed groups there was negative nitrogen balance. However, it was hypothesized that any weight loss by the RVP sheep would also be partially due to increased energy expenditure. In various

studies conducted by (Aquilani, et al., 2005; Bernstein, et al., 1997; Howard, et al., 1988),they suggest that either CHF experimental subjects or patients experience higher total energy expenditure, negative calorie and nitrogen balance, blood urea nitrogen and increased creatinine release. In contrast, this study did not find evidence for increased plasma NEFA and also palmitate turnover as seen by the previously mentioned investigators. In this light, it is possible to propose that during this study the catabolic and anabolic factors were not imbalanced to the degree necessary to cause significantly negative nitrogen balance in RVP sheep (e.g., skeletal muscle catabolic factors). In the present study, nitrogen balance was determined via urinary nitrogen excretion and a broader analysis of nitrogen-based compounds in plasma and urine (e.g., urea) may have been more reflective of the reduction in lean leg loss.

It would be expected that any loss of lean tissue would alter the A-V difference of amino acids plasma profile. Interestingly, there was elevated hind limb release of α-amino nitrogen via the iliac vein, and it is apparent from the DEXA analysis (Table 4.3) that this release accounts for the significantly lower lean leg mass in week 4 and week 8 of the experiment which agrees with a study by (Norrelund, et al., 2006). Infusion of intravenous amino acids may suppress amino acid catabolism in the hind limb as per (Chaloupecký, 1997). In addition, other authors have demonstrated significant changes in aterio-coronary venous difference of various plasma amino acids during CHF (Aquilani, et al., 2005; Brodan, et al., 1978; Engelen, et al., 2001). However, despite analysis of carotid and iliac α-amino nitrogen it may be more useful to examine the individual plasma amino acid (especially branched chain amino acids) profile and turnover which may yield more answers in regard to the lean tissue loss seen in this study. The addition of a third group with BCAA supplementation may also be useful to observe whether this treatment may assist to treat the lean tissue loss.

The present study showed no evidence of altered glucose profile (i.e., hyperglycaemia). This is in agreement with a study conducted by (Swan, 1994). However, CHF patients in that study had significantly higher mean fasting insulin concentration. On the other hand, the findings of the present

studies do not agree with those of (Suskin, 2000) who observed hyperglycaemia and hyperlipidemia in CHF pateints. Supporting this hypothesis, permanent RVP has been observed to be associated with modifications in glucose metabolism (Preumont, et al., 2005). Conversely, Beaufort-Krol et al. (1999), observed significantly lower arterial glucose concentration in lambs with aorta-pulmonary shunts, associated with decreased glucose production rate, glycogenolysis, and not through gluconeogenesis or hormonal control. Moreover, Norrelund et al. (2006) observed insulin resistance in CHF cachexia due to decreased glucose oxidation, which may be linked to left ventricular geometry (Leichman, et al., 2006). It may be suggested that the unaltered plasma glucose levels suggests that insulin production and sensitivity were unaffected in this model of RVP. In a further study of this model it would be advantageous to perform analysis for insulin, and a glucose tolerance test (GTT) measurement, which may further characterize glucose and insulin metabolism in this model.

Altered palmitate turnover has been demonstrated in CHF (Lommi, et al., 1998). Interestingly, (Lommi, et al., 1998), observed significantly higher FFA turnover and FFA oxidation in following an overnight fast human subjects experiencing CHF. However, in the present study there was no change in adiposity. Both (Lommi, et al., 1998) and (Norrelund, et al., 2006), observed elevated FFA levels (33.3% increase) in conjunction with elevated fat oxidation and muscle fat utilization. In addition, (Aquilani, et al., 2005), observed that at rest, arterial FFA concentration as well as FFA release were higher in CHF than in control patients Murray et. al., (2004) observed abnormal energetic activity in heart failure patients that correlated inversely with plasma FFA concentrations. The present study did not show any changes in plasma NEFA or palmitate turnover as there was no change in overall body adiposity. However, there was accumulation of visceral adiposity.

The present study demonstrates clearly that RVP at 180bpm for 8 weeks causes elevated energy expenditure. Further, (Lommi, et al., 1998), observed higher rates of energy expenditure in the CHF patients than in the non-CHF patients. Interestingly, the correction of anaemia in CHF cachexia also elevates REE and tissue oxygenation (Vaisman, et al., 2004). Moreover, there is a trend towards

increasing UCP 3 in skeletal muscle (+8%) in the RVP group. Various studies have shown a link between plasma FFA and elevation of UCP's in different tissues (e.g., UCP2 mRNA in heart) thus explaining the increase in energy expenditure (Guo, et al., 2006). Overall, increasing trend for UCP3 mRNA expression seen in the skeletal muscle samples in this study may result from reduced oxidative damage. Dietary intake of enzyme Co-Q10 or selenium may prove to decrease UCP2 mRNA and cause oxidative damage during RVP induced CHF (Littarru & Tiano, 2007);, possibly rectifying any lean tissue or weight loss observed in this study. In a future study there is a need to analyse all major tissues which contribute to energy expenditure (i.e., hepatocytes) for UCP and other proteins that regulate energy expenditure, and thus explain the elevation in energy expenditure seen in this model. The results of the present study relate to the characterization of a novel model of RVP induced cachexia development in sheep, as those proposed in cancer cachexia (Rosenthal & Musaro, 2002).

The limitations of analysis in this study include the ceasing of the pacing and weight measurements before a significant change in body weight could be observed. The study could have been continued until a significant difference could be observed and wasting established in this model. Also an increased number of animals would have assisted to decrease the standard error in body weight measurements. DEXA could have been confirmed with the use of chemical carcass analysis. Further,the inter-organ amino acid analysis would have yielded vitial information regarding the BCAA and other specific amino acids which may be altered during weight loss associated with CHF. A glucose tolerace test would have indicated the presence of type II diabetes. Muscle biopsy of wk 0, wk4 could have shown the trend in UCP2 and also liver analysis of UCP2 and UCP3 might have provided information about energy expenditure changes in the liver. Other genes that regulate fatty acid synthesis and catabolism could have also been analysed and add to the understanding of fat catabolism in Cachexia. The analyses mentioned would be necessary to provide extra information for publication.

In conclusion, the present study shows that RVP for 8 weeks, reduces body weight via induced leg muscle atrophy and elevated energy expenditure and this study has provided the basic framework

for the characterization of RVP induced CHF cachexia of a large animal model. There is further investigation needed using this model to examine abnormalities in protein and energy expenditure in cardiac cachexia.

CHAPTER 4

Effects of [1-Sarcosine] – Angiotensin II Infusion on Body Weight and Composition in the Rat

4.1 Introduction

Recent research suggests that CHF is best characterized as a dynamic disorder of many organ systems; including the myocardial, neuro-hormonal, immune, vascular, gastrointestinal, renal and musculoskeletal systems (Azhar, 2006). Previous researchers have characterized CHF with a high proportion of patients experiencing intense wasting, known as cachexia (Toth & Matthews, 2006). The pathogenesis of cardiac cachexia differs from anorexia as there is an increase in energy expenditure and skeletal muscle wasting despite calorie and protein intake. Overall there is a general lack of suitable animal model (Busquets, et. al., 2004; Héliès-Toussaint, et al., 2005), and thus need for their development. The majority of studies examining energy expenditure in cachexia has been conducted in humans and offers limited understanding of specific mechanisms involved (Poehlman, et al., 1994; Toth, et al., 1997; Toth, et al., 2006).

Moreover, Brink, et al., (1996) and Brink et al. 2001 reported a rat model of Ang II infusion induced CHF which also resulted in weight loss. IGF-I was implicated as a key mechanism involved in the development of CHF in this model of wasting (Brink, et al., 1996). In support, numerous experiments (Brink, et al., 2001; Porter, et al., 2003; Song, et al., 2005) detail a possible mechanisms causing the weight loss. The mechanisms suggested were decreased circulating IGF-I, causing decreased lean mass and increased protein degradation via blockage of the autocrine IGF-I system. In addition, (Cassis, et al., 1998) also observed decreased circulating levels of IGF-I, but documented unchanged feed intake, and increased blood pressure. In addition, they also detected a rise in thermal

infrared abdominal surface temperature and decreased white adipose tissue indicating to elevated energy expenditure based on an Ang II dose-based relationship for weight loss. Further, (English, 1999) hypothesized that increased sympathetic neurotransmission in interscapular brown adipose tissue is a possible mechanism of weight loss. Moreover, (Porter, et al., 2003) induced CHF and weight loss using Ang II infusion and concluded that the weight loss was attributable to reduction in feed intake and an independent component (i.e., IGF I or UCP 1 thermogenesis). However, to date, no studies have effectively investigated the exact origin or the function of proteins responsible for the elevated energy expenditure. The study described in the previous chapter (Chapter 3) validated the use of RVP pacing in a large animal model to test whether there was evidence of wasting and weight loss. The present study used Sarcosine-Angiotensin II (*N*-methyl glycine-Angiotensin II) to induce CHF wasting instead of Ang II due to it being a potent analogue, which has a higher affinity for the AT1 receptor than Ang II (Bihoreau, et al., 1993; Cordopatis, et al., 1994) thus potentiating its effect. In the present study, the degree of wasting was observed and if there was an elevation in energy expenditure with adult female Sprague Dawley rats being infused to SAR-Ang II for a period, and then observed for catch up growth during a recovery phase.

Porter, et al., (2003) suggested a role of UCP1 in BAT (i.e., thermogenesis), but this did not explain elevation of energy expenditure in other tissues, particularly the liver (i.e., site for controlled energy expenditure and cross-talk with adipose tissue) (Uno, 2006). Thus, investigation of UCP levels in these tissues needs further attention. Evidence has arisen which shows that UCP's may have a role in cachexia (Bing, et. al., 2002; Bing, et al., 2006), and thus are candidates for the unexplained increase in energy expenditure. The principle hypotheses of the present study is that exposure to SAR-Ang II results in the development of body weight loss of both adipose tissue and skeletal muscle. Further, this wasting is caused by increased energy expenditure. Increased energy expenditure is a result of UCP 2 and UCP3. UCP2 is a marker of inefficiency in mitochondrial energy production and regulator of ROS (Chu & Leung, 2007). Wasting in rats was measured using

body weight and end-point body composition and metabolite measurements. It is hypothesed that in this study, infusion of AngII for a 5 day period would induce CHF Cachexia via increased energy expenditure caused by UCP2 in the skeletal muscle and that the rats will recover in the post-infusion stage

4.2 Materials and Methods

4.2.1 Animals

All rats used in the experiments were adult females of the Sprauge Dawley strain. The initial weight range for the animals was 250 – 350g. The rats were sourced from the Dept. of Zoology, The University of Melbourne, Melbourne, Australia. Prior to experimentation, animal ethics committee approval was granted for the experimental procedures (La Trobe University) (01/22L). Thirty three rats in total were used for the experiment (11 pair-fed control, 12 *Ad libitum* and 10, [1-Sarcosine]-Angiotensin II infused rats). Four days prior to surgery the rats were placed in metabolic cages so they could acclimate to their surroundings. The [1-sarcosine]-Ang II infused rats were fed *ad libitum* and the sham operated control rats were pair-fed after the performance of initial surgery. Daily measurements of body weight, faecal excretion, feed intake (g), volume of water consumed (mL) and the volume of urine excreted (mL) were recorded. The measurements were recorded in three different periods (4 days prior to surgery – '*Pre-infusion*'; day of surgery and five days after surgery – '*infusion period'*, and day 7 to 14 – '*Recovery'* (Depicted as a 4, 6 and 8 day intervals) The faeces and feed were dried and weighed for further analyses to determine the energy content and moisture content as outlined in the general methods (Chapter 2) and in the sections below.

4.2.2 Housing

Throughout all studies, rats were maintained in metabolic cages (890 x 560 x 560 mm). The rats were housed at a constant 21^{o}C±1 ^{o}C environmental temperature.

4.2.3 Body Weight

Daily live weights for the rats were determined using a balance being measured to $1/100^{th}$ of a gram.

4.2.4 Food and Water

The rats were fed a range of feeding regimes. [1-sarcosine] - Ang II rats were allowed to eat *Ad lib*, controls were pair-fed to the [1-sarcosine] - Ang II group and an additional *Ad lib* control group. The diet was a barastoc rat chow ration (Ridley Agriproducts Pty Ltd. Vic. Aust.).
The rats were supplied with an *Ad libitum* water supply. Both feed and water intake were measured on a daily basis.

4.2.5 Faecal and urine collection

Urine was collected under the metabolic cage into a volumetric cylinder containing 25µl of concentrated sulphuric acid to reduce ammonia loss. Faeces were collected into a tray below the metabolic cage. Both urine and faeces were frozen at $-20^{o}C$ until analysis was performed.

4.2.6 Surgery and Osmotic Mini Pump Implantation

Before surgery, the operating table was washed with 70% ethanol and all apparatus needed were autoclaved (dry). Each osmotic mini-pump (Alzet model 2001) was filled with 200µl of SAR-Ang II. The SAR-Ang II was infused at a rate of 500ng/kg live body weight/min (1.0µl of infusate per hour ± 0.03µl). The Angiotensin was dissolved in saline that was acidified with 0.01M acetic acid. The solution was then pumped through a 0.22µM filter to ensure the sterility of the infusate. The pump was filled using a blunt end syringe, and then the flow modulator inserted. After the pump was prepared the rat was initially given anaesthesia. The rat was allowed to rest for 5 minutes before the anaesthesia took

effect. The rat was laid on its stomach and the hair above and below the scapulae was shaven (~2cm each way). The surgical area was swabbed with 10% hibitaine, and then betadine (1% w/v iodine). Initially, the rat was tested for pain sensation by pinching the tail. If no response was evident the surgery proceeded. An incision was made adjacent to the site chosen for pump placement (mid-scapular). A haemostat was inserted into the incision site under the skin, with the subcutaneous tissue being spread-opened using the jaws of the haemostat, creating space for the pump. The filled pump was inserted into the incision site. It was inserted with the flow modulator facing away from the incision site and the wound closed using Michel clips (10 x 3mm) and a suture (2.0 Dexon). The rat was allowed to rest on a heat pad until it recovered from the effects of anaesthesia.

4.2.7 Anaesthesia

Prior to surgery rats were administered either an intra-peritoneal (lower left quarter) injection of anaesthetic (60mg/kg Ketmine (100mg/mL) and 12mg/kg Xylazine (20mg/mL) or 150µl of a 5mg/mL intra-muscular injection (i.e., hind-limb) of Zoletil. A 23 gauge x 1” needle with 1mL syringe was used for anaesthesia administration.

4.2.8 Catheterisation

Rats were anaesthetized as previously mentioned (section 4.2.7.). Once the animals were under the influence of the anaesthesia the ventral midline was cut open using surgical scissors, exposing the peritoneal cavity. The descending aorta and inferior vena cava (iliac vein) were separated via teasing the membrane attaching them with a hemocrit. A 23 gauge needle x 1” needle with tubing (100mm length) attached to a 1mL syringe was used to withdrawal blood. Next the portal vein was located and a curved 23 gauge x 1” needle was inserted at least 1cm into the vein. Lastly the renal vein was located by removing peri-renal adipose tissue and again the needle was inserted 1cm into the vein. The venous

blood was collected very slowly with little pressure as to avoid contamination of arterial blood crossing organ beds.

4.2.9 Blood and organ sample collection

Blood samples (1mL each) were obtained from a various vessels; portal vein, iliac vein, renal vein and an arterial blood sample of 2mL from the descending aorta. The samples were collected into syringes containing 5µl of 2500 IU/mL (12.5U total) of heparin. The openings to the vessels were closed using a drop of adhesive glue, and the organs removed to be weighed; liver, kidney, heart, spleen, lungs, and carcass. The blood was immediately centrifuged at 8000 *g* for 15 mins at 4°C to obtain plasma. The plasma was stored at -70°C to await further analysis. In addition, tissue samples of skeletal muscle, heart, spleen, liver and adipose tissue were placed in 1mL RNAlater, and then frozen at –70^{o}C. These samples were used in mRNA analysis. After the blood and organ samples were collected the rat was culled by excision of the heart.

4.3 Analytical Methods

4.3.1 Carcass Homogenization and moisture content

Whole carcasses were emptied of their viscera and the major organs were weighed. The carcass was weighed and then frozen at –20^{o}C. The carcass and organs were diced frozen into small cubes (10 x 10 x 10mm approx.) and then homogenized twice using a meat grinder (Porkret, Czek Rep.) (2mm die cast size). A sub-sample of each rat homogenate was then dried at 60^{o}C until weight became stable to determine moisture content. The dried carcass powder was then sealed in an airtight plastic bag and stored at –20^{o}C pending further analysis.

4.3.2 Organic Matter Determination, Ash and AIA

For organic matter determination, ashing and AIA methods please refer to section 2.7.7. and 2.7.8. respectively in Chapter 2.

4.3.3 Kjeldhal Nitrogen (Crude Protein)

Crude protein for body composition of the rat carcass was determined using the Kjeldhal method. The dried homogenate was ground into a powder and approximately 0.5g was used for protein analysis. For details of this procedure please refer to section 2.7.2 in Chapter 2.

4.3.4 Adipose Tissue Determination using Soxhlet apparatus

The dried homogenized carcass tissue was placed in filter paper (Whatman 541), folded and fastened with a paper clip. The parcel was then placed in an oven for 24hrs at 70^{o}C to remove residual H_2O and placed in a desiccator. The parcel was weighed and then placed in a soxhlet apparatus. The solvent mixture used for fatty acid extraction was absolute chloroform: absolute methanol (2:1). In an optimization experiment, an 8 hour extraction period was sufficient to remove all fat from the parcel.

The co-efficient of variation was 8.16% for this method. The variability may be due to insufficient homogenizing of carcass tissue rather than accuracy of the method.

4.3.5 Blood Metabolites

For details of methods concerning α-amino nitrogen, and NEFA please refer to section 2.7.3 in Chapter 2.

4.3.6 Energy Expenditure per period

The energy expenditure per period was calculated using the following equation;

Energy Expenditure = Food Intake *(MJ)* – (Final Carcass *(MJ)* / Initial Carcass *(MJ)*)

4.3.7 Uncoupling Protein mRNA expression

For details of method used for the analysis of UCP2 and UCP3 mRNA expression, please refer to section 2.9 in Chapter 2.

4.4 Results

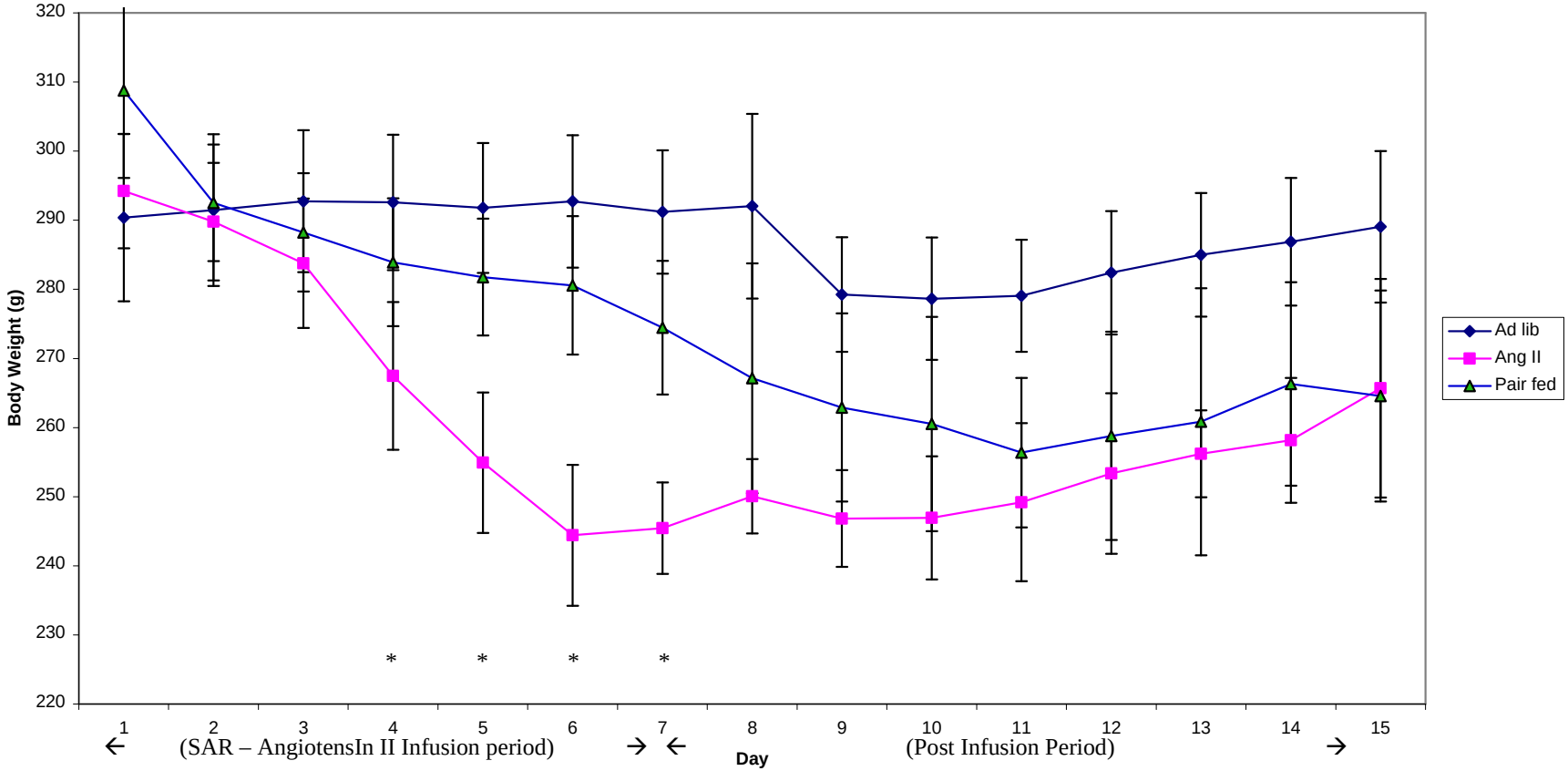


Figure 4.1 Body Weight (Day 1 – 5; Infusion Period) and (Day 6 – 14; Post Infusion Period) of SAR-Angiotensin II (200ng/Kg/d) (Ang II), Pair-Fed and Controls. Values are presented as means ± standard errors. Control vs. treatment *p<0.05.

Initial weights were similar in all groups. During the infusion period, the SAR-Ang II group displayed rapid weight loss ($p<0.05$) compared to the *Ad libitum* and pair-fed groups. During the post-infusion period, the Ang II group's body weight gradually increased from day 10 in line with the pair-fed group, however there was no significant difference. This re-gain may have been due to catch-up growth (Figure 4.1).

The SAR-Ang II group displayed lower ($p<0.05$) % fat content when compared to the control group. There were no other differences observed in any of the body compositional parameters displayed in Table 4.1, thus the weight change was consistent over the composition of skeletal muscle and adipose tissue portion of the carcass in respect to the pair-fed group. However, the infusion of Ang II into the rat rapidly produced a significantly negative body weight change (g/d) as outlined in Table 4.1, in comparison to the pair-fed and control groups. When energy expenditure was expressed per gram of body weight the Ang II rats had a siginifacnt higher expenditure than both the control and pair-fed rats. However, when expressed per gram of skeletal muscle the Ang II group was significantly higher ($p<0.05$) than only the pair fed group.

Ang II infusion resulted in negative body weight change ($p<0.01$) (both per gram % of initial body weight) (Table 4.2) and per (g) body weight, in comparison to both the pair fed and control rats, and further decreased carcass adipose tissue in comparison with the control which did not further decrease. Thus, changes in body composition were related to calorie restriction and not the effects of SAR-Ang II; however, an additional component of pair-fed independent weight loss is yet to be elucidated. Energy expenditure on a per gram basis was increased ($p<0.05$) in the SAR-Ang II group compared with the control and pair fed and when expressed on a per gram body protein only with the pair-fed group (Table 4.1).

Table 4.1 Body Composition at end of infusion period. Energy expenditure expressed as g of body weight and g of body protein.

	End-Infusion Period		
Carcass Component	**Control**	**Ang II**	**Pair Fed**
Ash (Bone)%	5.5±1.3 (4)	7.5±0.89 (4)	7.7±0.83 (4)
Fat %	46.4±3.13 (4)	25.1±3.91 (4) ‡	33.1±2.59 (4)
Protein content (Muscle) %	53.5±3.4 (4)	60.8±2.2 (4)	60.2±1.2 (4)
Carcass GE (MJ/kg)	25.5±3.4 (4)	22.9±0.63 (4)	24.4±0.59 (4)
Carcass Water %	60.75±1.33 (4)	59.91±1.17	59.29±0.75
EE per g body weight (MJ)	0.007±0 (4)	0.009±0.001 (4)* ‡	0.006±0.001 (4)
EE per g body protein (MJ)	0.013±0.001 (4)	0.016±0.001 (4)*	0.01±0.001 (4)

Results are presented as means ± standard errors. ‡Ang II vs. Control. *Ang II vs. Pair-fed $p<0.05$
N.B. The protein % includes a proportion of the Carcass Water %

Table 4.2 Initial, final body weight, feed intake, and change in tissue per period – infusion period

	End-Infusion Period		
Carcass Component	**Control**	**Ang II**	**Pair Fed**
Initial Body Weight (g)	292.4±11.7 (8)	292.4±8.1 (5)	299.6±11.9 (7)
Final Body Weight (g)	294.7±9.4 (8)	244.9±11.4 (5)*‡	277±9.2 (7)
Change in Body Weight (g)	2.34±3 (8)	-47.41±8.01 (5)*‡	-22.54±4.7 (7)*
Change in Body Weight (%)	1.0±1.1 (8)	-16.3±2.9(5)*‡	-7.3±1.3 (7)*
Feed intake in Period (g)	100.43±3.91 (8)	55.1±8.87 (5)*	55.66±6.45 (7)*
Water Intake (mL)	40.3±3.5 (8)	45±5 (5)	29.3±6.45 (7)

Results are displayed as mean ± standard error (number of observations). * signifies $p<0.05$ (Ang II vs. Control) and ‡ signifies $p<0.05$ (Ang II vs. PF)

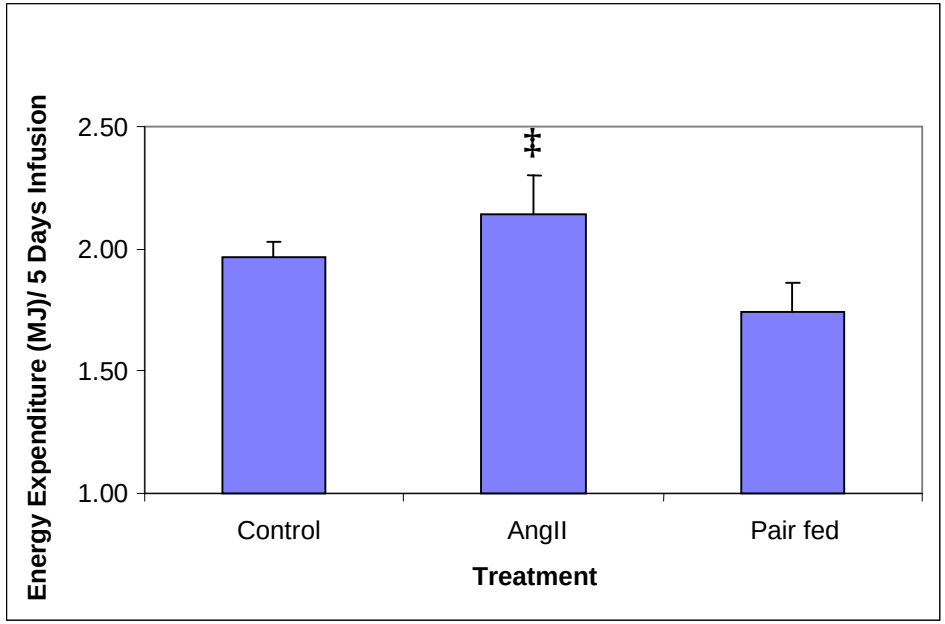


Figure 4.2 Total Energy Expenditure during the infusion period; Ang II, Pair - Fed and Controls. Values are presented as means for the four animals ± standard error. ‡ Ang II vs. Pair-fed $p \leq 0.05$

Table 4.3 Organ weights (g). Results are displayed as mean ± standard error (number of observations).

Organ	Infusion Period			Post-Infusion Period		
	Control	Ang II	PF	Control	Ang II	PF
Liver	8.52±0.69 (4)	9±0.69 (4)	9.1±0.69 (4)	8.52±0.69 (4)	10.3±1.05 (4)	8.9±1.05 (4)
Kidney	2.1±0.41 (4)	2.02±0.1 (4)	2.2±0.1 (4)	2.1±0.41 (4)	2.4±0.15 (4)*	1.9±0.15 (4)
Heart	0.86±0.82 (4)	0.95±0.06 (4)	1.0±0.06 (4)	0.86±0.82 (4)	0.9±0.09 (4)	0.89±0.09 (4)
Spleen	0.5±0.55 (4)	0.54±0.06 (4)	0.54±0.06 (4)	0.5±0.55 (4)	0.79±0.1 (4)	0.46±0.1 (4)
Lungs	1.07±0.18 (4)	1.23±0.14 (4)	1.68±0.14 (4)	1.07±0.18 (4)	1.42±0.21 (4)	1.33±0.21 (4)
Carcass	257± 6.24 (4)	203±10 (4)*‡	224±20.2 (4)*	266±6.4 (4)	210±10.6 (4)*	220±28.5 (4)*

*Control vs. treatment $p<0.05$ and ‡ Ang II vs. Pair-Fed $p<0.05$

As seen in Figure 4.2, the estimated energy expenditure during the infusion period for the SAR-Ang II group is higher ($p<0.05$) compared with the pair-fed group. From Table 4.3, the SAR-Ang II group displayed lower body and carcass weight (g) during the infusion period when compared with both the control and pair-fed groups. The pair-fed group displayed lower ($p<0.05$) carcass weight in comparison to the control group. However, in the post infusion period both the SAR-Ang II and pair-fed group displayed lower ($p<0.05$) carcass weight in comparison to the control group.

The effect of SAR-Ang II infusion on organ weights is shown in Table 4.3 and Figures 4.3 – 4.5. The weights of liver, kidney and heart expressed as a percentage of body weight are presented in Figures 4.4 and Figure 4.5 and 4.6 respectively. In the infusion period, the kidney was the only organ in the pair fed group to display lower mass (g) ($p<0.05$) when compared with the control group. However, when the organs were expressed as a percentage of body weight (Figure 4.4 and 4.5) the liver in the SAR-Ang II group was higher ($p<0.05$) in the infusion period when compared with the pair-fed and control group and both the pair-fed and SAR-Ang II group had a larger ($p<0.05$) liver mass as a percentage of body weight when compared with the control group in the post-infusion period. Moreover, the kidney as a percentage of carcass weight was larger ($p<0.05$) in the SAR-Ang II group in both the infusion and post infusion period in comparison to both the pair-fed and control group. Lastly, during the infusion period, the heart weight as a percentage of carcass weight was larger ($p<0.05$) in both the SAR-Ang II and pair-fed group when compared to the control group.

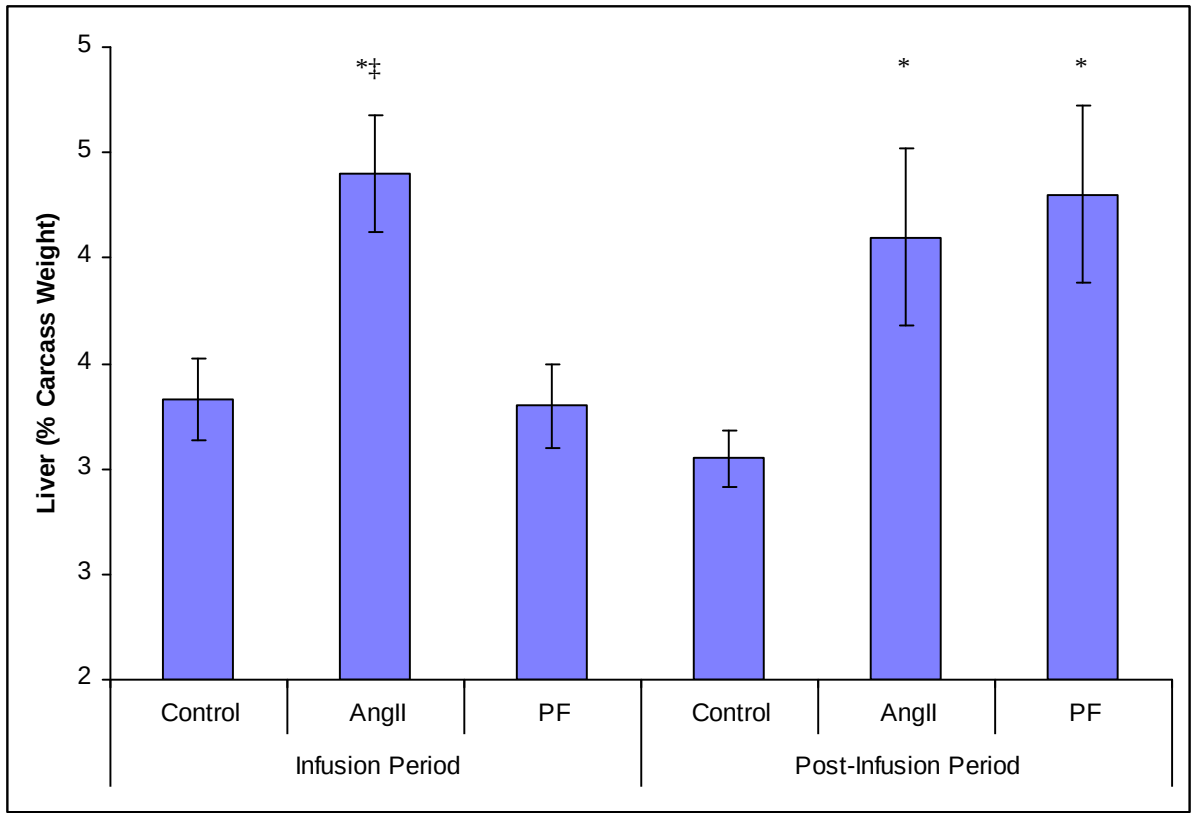


Figure 4.3 Liver weights as a percentage of total carcass weight. Values are presented as means for ± standard error. *Control vs. treatment $p<0.05$ and ‡ Ang II vs. Pair-Fed $p<0.05$

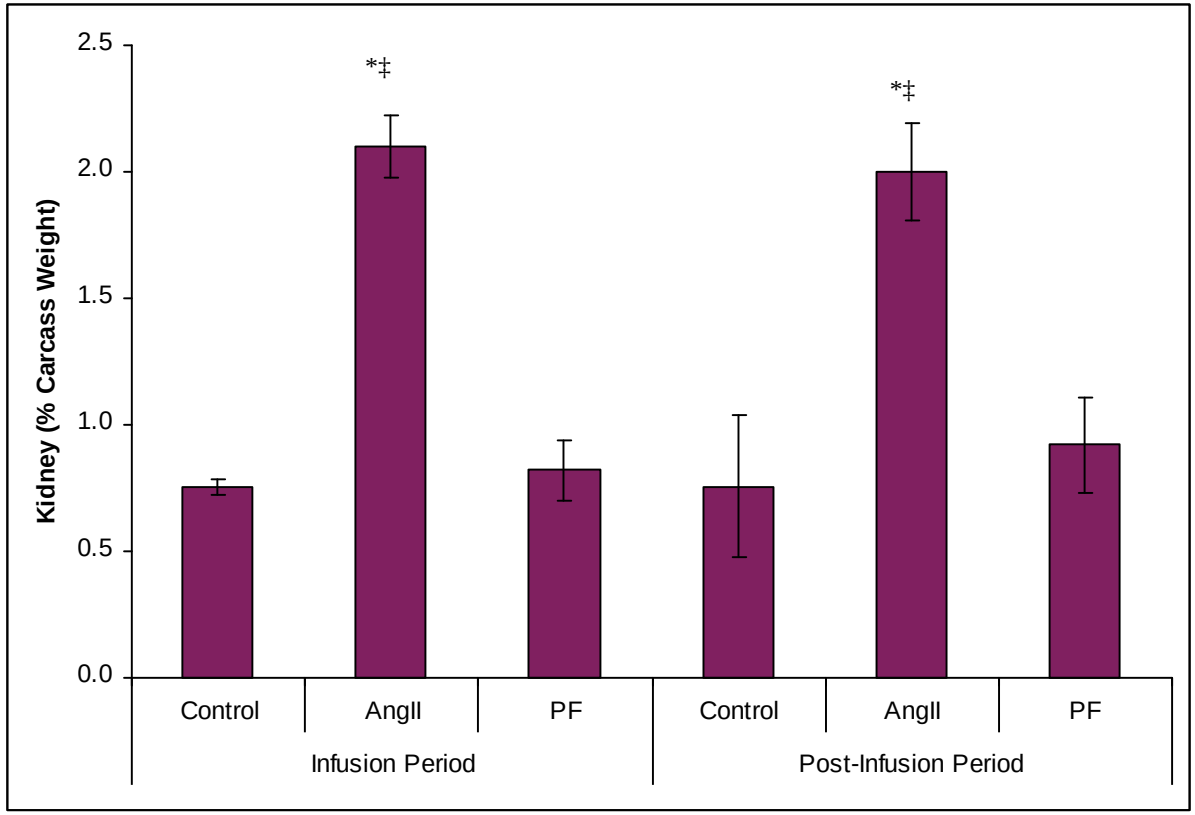


Figure 4.4 Kidney weight as a percentage of total carcass weight. Values are presented as means for ± standard error. *Control vs. treatment $p<0.05$ and ‡ Ang II vs. Pair-Fed $p<0.05$

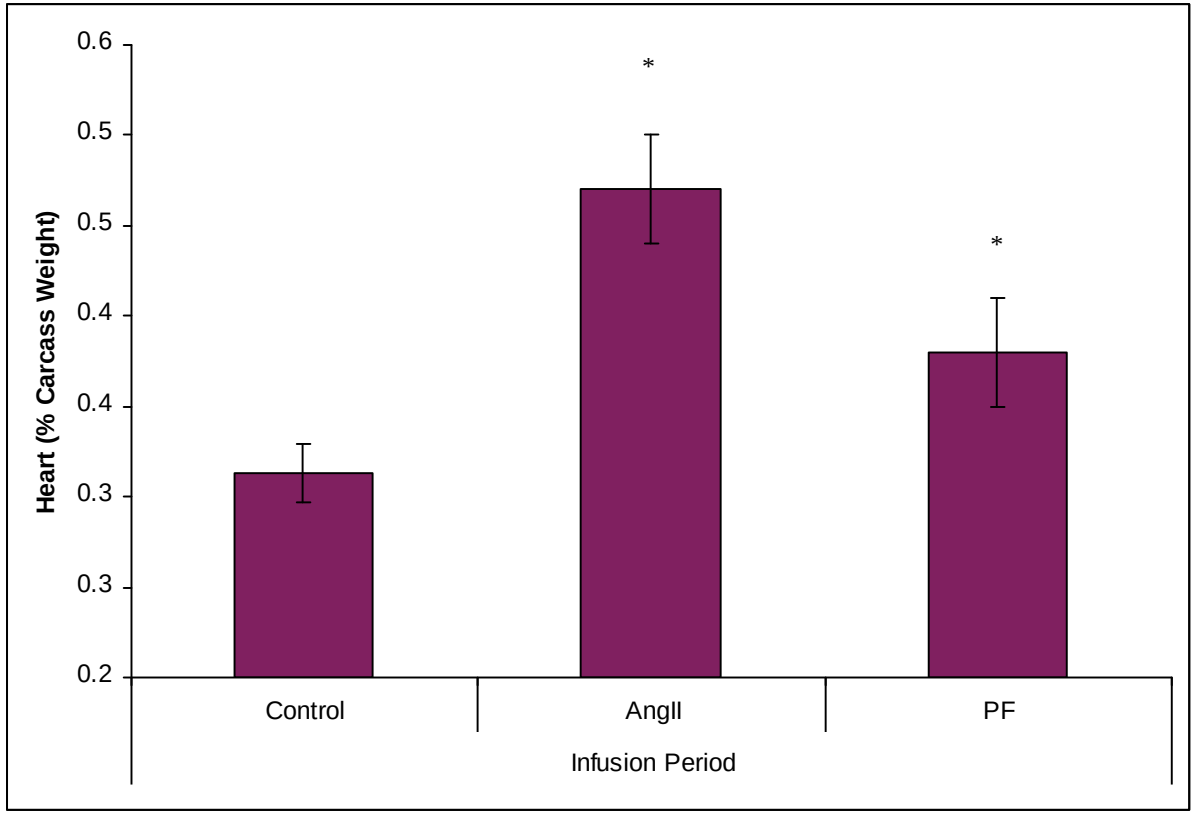


Figure 4.5 Heart weight as a percentage of total carcass weight. Values are presented as means for ± standard error. *Control vs. treatment $p<0.05$ and ‡ Ang II vs. Pair-Fed $p<0.05$

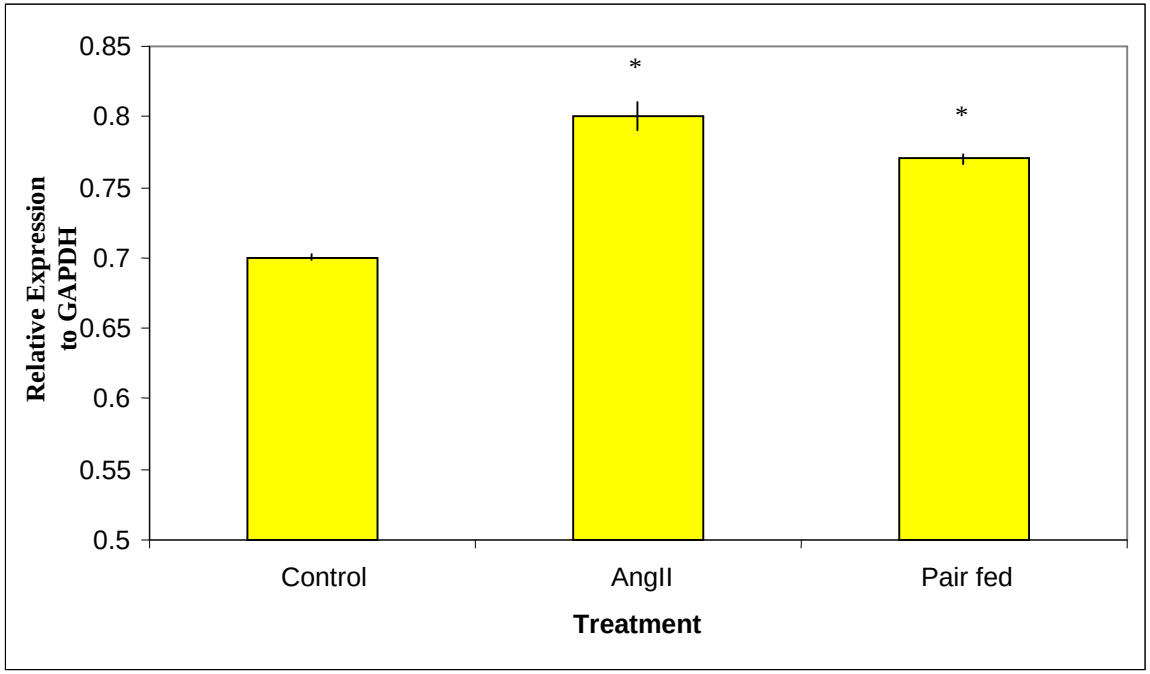


Figure 4.6 Uncoupling Protein 3 (UCP3) (Skeletal Muscle) of Control, Angiotensin II (Ang II), PF, relative to the mRNA expression of the house-keeping gene (GAPDH)
Signifies $p<0.05$ (Ang II vs. Control) and ‡ signifies $p<0.05$ (Ang II vs. PF)

When examining Figure 4.6, it is apparent that there is increased ($p<0.05$) UCP3 mRNA expression in the SAR-Ang II group and Pair-fed group in comparison to the Control group.

4.5 Discussion

The purpose of the present study was to investigate mechanisms of wasting caused by SAR-Ang II infusion in the female Sprague Dawley rat. To further extrapolate out the effect of SAR-Ang II on metabolism, energy expenditure was determined from feed intake and carcass composition and the levels of UCP 3 mRNA were determined in skeletal muscle. Initially, body weight was used as a measure of wasting. However, more invasive techniques were required to examine the carcass component affected by wasting.

It was hypothesized that infusion of SAR-Ang II would cause noticeable weight loss, partially due to reduced feed intake which was also seen in the pair-fed group, but a newly identified component of weight loss was observed. This portion of weight loss is independent of anorexia as seen during the infusion period, and contrasts with body weight changes in the pair-fed group. The weight loss was balanced between all three components of body composition; muscle, fat and bone. This is a highly significant result and suggests that a component of weight loss may be attributed to elevated metabolic activity. Decreased body weight agrees with the majority of studies utilizing Ang II infusion to induce weight loss and has generally been attributed to loss of appetite, decreased food intake and, thus, lowered energy intake (Brink, et al., 1996; Porter, et al., 2003; Porter, et al., 2004). However, increased energy expenditure that may cause weight loss independent of anorexia needs further elucidation.

In the present study, there is evidence to suggest that a component of weight loss in the Ang II group may be due to increased energy expenditure. This result is agreeable with a study by (Porter, et al., 2003) who suggests that systemic infusion of Ang II decreases body weight and food intake via increased energy expenditure (i.e., thermogenesis, increased UCP1 mRNA expression in Brown Adipose Tissue), while (Porter, et al., 2004) also observed that inter-cerebral ventricular infusions of Ang II produced transient decrease in food intake. A decrease in food intake was also seen in this study. The relevance of this finding is that Ang II directly reduces feed intake and thus

affects body weight (i.e.,, via calorie restriction), yet there is an increase in energy expenditure on a per g body weight basis.

Interestingly, in the Ang II infused group the rats ate half as much feed as the *Ad libitum* controls, and still had greater energy expenditure (+20%). Even more striking is the fact that the pair fed control group had an average body weight of 277.9g and the Ang II group had an average body weight of 244.9g at the end of the infusion period or 11.8% less than the pair fed controls. However, the Ang II rats had a total energy expenditure of 22.7%. In an attempt to inversely correlate weight loss with increased energy expenditure, there is a difference of 10.9% exists, where this energy may have been dissipated due to a futile cycle such as increased activity of uncoupling proteins. This is a highly significant finding as calorie intake by CHF cachexia patients is not soley responsible for weight gain/loss, yet an independent factor is present. Thus, this metabolic factor could be a function of weight regulation and not a summative factor of body weight in cachexia as per calorie intake or physical activity.

Even more interesting, when the energy expenditure was expressed per gram of body weight, the Ang II rats had a siginifacnt higher expenditure than both the pair-fed and control rats (29.65% and 33.12% respectively). The same trend exists for the energy expenditure expressed on a per gram of body protein, however the percentages change (pair-fed 32.8% and control 17.4%) and the difference with the control group is not significant in comparision to the Ang II group. These findings suggest that a mathematical equation could be written to predict energy expenditure based on the body's percentage of skeletal muscle (inverse relationship). It would be assumed that the degree of adipose tissue would also impact on this relationship and it would not be entirely linear. This work requires further examination. Further, the pair-fed rats were only 11.8% heavier at day 5, however they had a skeletal muscle energy expenditure of ~33% less than the Ang II rats. These results directly suggest that the Ang II rats had skeletal muscle energy expenditure comparable to the *Ad lib* control group even though they ate 50% less. These measures are conclusive proof that Ang II directly elevates energy expenditure, particularly within skeletal muscle. The investigation

of genes involved in protein turnover and energy expenditure would add to the knowledge gained in this study and give greater insight into the regulation of energy expenditure in Ang II induced weight loss.

Although differences in energy expenditure between the groups of rats may contribute to the difference in degree of weight loss, other research suggests that Ang II acts directly on the brain (possibly through the AT1 receptor protein - (Porter, 1999) to affect food intake and energy expenditure in a manner not related to water intake, as water intake was increased in the SAR-Ang II rats, contradicting (Cassis, et al., 1998). In the first few days of infusion there was indeed an increase in water consumption, but steadily followed with diminished food intake.

It was also hypothesized that, due to increased energy expenditure, there would be a large degree of skeletal muscle atrophy, yet also adipose tissue loss. In contrast to the control group rats, the Ang II rats did display decreased carcass adipose tissue, possible due to reduced (~50%) feed intake. However, the degree of adipose tissue loss may not be explained solely using the hypophagia hypothesis. These findings agree with Cabassi, et al., (2005) who postulated the hypothesis, that Ang II to be a regulator of lipid metabolism as after Cabassi, et al., (2005) infused Ang II in rats for 12 days results in higher sustained interstitial glycerol. The higher norepinephrine levels, stimulated reduced adipocyte diameter in subcutaneous and visceral (retroperitoneal and epididymal) fat tissues, possibly via sympathetic activation and beta-adrenergic-receptor stimulation. The results of the present study show that infusion of SAR-Ang II in female Sprague Dawley rats causes wasting of both adipose tissue and skeletal muscle, due to change in levels of catabolic hormones regulating these tissues.

The present study has found that infusion of SAR-Ang II does not specifically induce skeletal muscle wasting, but rather whole body wasting. Mechanisms to explain skeletal muscle wasting are proposed by (Brink, et al., 2002; Brink, et al., 1996; Delafontaine, et al., 2000; Song, et al., 2005) who observed a link between the activation of the renin-Angiotensin system. Further, it has been observed that skeletal muscle-specific wasting is due in part, due to decreased levels of

circulating and skeletal muscle IGF-I, while Song, Li et al. (2005) solely observed decreased activated caspase-3 and ubiquitin ligases atrogin-1 in skeletal muscle. These studies focused more on skeletal muscle than adipose tissue metabolism. Lastly, (Delafontaine & Akao, 2006) suggest that Ang II plays more of a role in muscle wasting leading to cardiac cachexia and that IGF-I is a key candidate in the pathway of wasting. The development of wasting and cardiac cachexia in this study was characterised by an equal decrease in total body weight, adipose and skeletal muscle tissue.

The reduction in body weight can be partially attributed to reduced feed intake. This has been observed previously by (Cassis, et al., 2002) who observed hypophagia in Ang II infused rats. The hypophagia accounted for 63% of the effect of Ang II on body weight, yet the AngII infusion did not influence systolic pressure, water intake, or oxygen consumption. The results of the current study support the findings of (English, 1999)**,** who proposed a weight loss effect attributable to the actions of elevated noradrenaline (NE) release from inter-scapular brown adipose tissue sympathetic nerve terminals. This weight loss is also associated with lipolysis as Ang II infusion invokes higher sustained interstitial glycerol and norepinephrine levels (Cabassi et. al. 2005). Consistent with a number of studies by (Cassis, et al., 1998) and (Weisinger, et al., 2007), increased energy expenditure displayed in Figure 4.3 and elevated UCP3 seen in Figure 4.9, contribute to weight loss. This increased energy expenditure may involve action by hormonal (NE, IGF-I, leptin) and cellular signalling (UCP's) leading to decreased adipose and skeletal muscle tissue. The regulation may arise from the mitochondria to influence catabolism of skeletal muscle and adipose tissue, supplying much needed substrate for elevated energy expenditure.

The elevated energy expenditure seen in the Ang II group agrees with the findings of other authors (Toth, et al., 1994; Toth, et al., 2006), who all observed elevated energy expenditure in CHF and CHF cachectic patients. This elevated energy expenditure could be attributed to a combination of elevated hormonal levels (i.e., leptin and epinephrine). Porter and Potratz (2004), showed that intra-cerebroventricular infusions of Ang II increased energy expenditure in older rats as evidenced

by increased UCP-1 mRNA expression in BAT (i.e., elevated thermogenesis), but may be due to other metabolic regulatory factors possible (i.e., UCP 2 or UCP3. Moreover, (Cassis, et al., 1998) observed that with the use of thermal infrared imaging, increased abdominal surface temperature in Ang II infusion, being possibly due to thermogenesis in WAT and BAT contained in the viscera. In addition, (Cassis, et al., 2002) , observed that oxygen consumption was transiently decreased on day 1 of Ang II infusion followed by a rebound increase over a 28 day period. It would be expected that the increased energy expenditure would have increased oxygen demand in line with (Cassis, et al., 2002).

(Poehlman, et al., 1994) observed resting metabolic rate to be ~18% higher in CHF patients than in pair fed controls when indexed per kilogram of fat-free mass. This finding supports the hypothesis that reduced energy intake does not translate to reduced energy expenditure and, in fact, that energy expenditure increases causing negative energy balance and weight loss from both skeletal muscle and adipose tissue. This finding and the supporting hypothesis negate other observations which suggest that the suppression of feed intake may also counter-act the elevated energy expenditure leading to potentially lower energy expenditure than upon re-feeding (Forman-Hoffman, 2006)

It was proposed that the heart weight in the SAR-Ang II group would increase with infusion time and indeed it was observed in this study ($p<0.05$) compared with the pair fed and control group. This finding is supported by an observation by Huentelman, et al., (2005) who showed that infusion of 200 ng kg^{-1} min^{-1} Ang II for 4 weeks resulted in a significant increase in the heart weight to body weight ratio. Further, the liver hypertrophy seen in the Ang II group agrees with Belin, et al., (2006), who observed that liver weight is increased ($p<0.05$) during left ventricle myofilament dysfunction in the rat (Brink et al. 1996).

Moreover, the increasing trend of UCP's mRNA expression in skeletal muscle via systemic infusion of Ang II agrees with findings by Porter et al. (2003), who observed decreased body weight was due to elevated energy expenditure in BAT (i.e., elevated mRNA expression of UCP1;

thermogenesis UCP in BAT) (Porter et al. 2003). Although UCP1 was not measured in the present study, UCP3 has been described to be expressed in skeletal muscle and WAT and thus be a more direct measure of 'metabolism leak' in these tissues. Ang II group experienced underfeeding, which led to an increase rather than a decrease in REE, with a parallel increase in UCP3 in skeletal muscle. The human skeletal muscle is a significant site of whole-body energy expenditure in lean individuals. It has been observed that there is no significant change in UCP3 mRNA levels in skeletal muscle pre- and post-under feeding (Seevaratnam, et al., 2007). Another UCP's or an energy futile cycle may be influencing energy dissipation (e.g., glycolytic pathway influencing proton leak) (Beauvoit, et al., 1993).

The role of UCP's has been documented in other forms of wasting (Bing, et al., 2000; Busquets, et al., 2005) and the total weight loss observed may be attributed to the increased UCP activity. Further investigation is needed to identify tissue specific elevations in UCP's. Further, high plasma glucose and Ang II lead to significant ROS production. In turn UCP-2 mRNA expression is elevated and reverses these effects and thus regulating intracellular ROS production (Park, et al., 2005). This implies that the role of UCP's in chronic disease is detoxification of free radicals (Sanders, 2004). This observation is further supported by Guo et al. (2006) who observed that UCP 2 mRNA is upregulated 2.8 fold in CHF and to counteract ROS generation. Lastly, the liver is a major site of energy expenditure and thus UCP 2 expression (e.g., liver and SKM proton leak of the rat accounts for 15-20% of BMR (Rolfe, et al., 1997; Rolfe, et al., 1999), however during this study there was too much variation in results for liver UCP2 and hence this analysis was not reported and should be repeated with a higher number of observations.

In summary, the present study demonstrated that infusing of SAR-Ang II impairs appetite, and reduces body weight via wasting that may be partially explained by elevated energy expenditure possibly induced by UCP. This finding is akin to previous reports from rat studies (Porter, et al., 2003; Porter, et al., 2004; Rolfe, et al., 1997; Rolfe, et al., 1999). The wasting proceeds in a cumulative daily dosage and when infusion of AngII ceases there is some degree of

weight re-gain. There are many actions of SAR-Ang II that could have contributed to the wasting in this model of CHF, however not all mechanisms were fully investigated in this study. One mechanism to mention is that UCP's may be one link in a complex cascade which governs energy expenditure and thus weight loss in chronic illnesses such as CHF. Thus, in order to increase the certainty of conclusions, all future studies must use this study as a guide and endeavor to examine all possible mechanisms that may underlie energy expenditure using gene expression analysis. In conclusion, this study highlight the need for continued use of this model to determine origin of elevated energy expenditure and key regulators of specific body weight components and possible treatment methods.

All limitations mentioned were caused by lack of funding and time.The limitations of this particular study include not being able to confirm body composition measurements using DEXA, the inability to measure region carcass composition using DEXA and thus observe any specific region of the carcass which wastes faster (e.g. hindlimb). Also to continue the post infusion period until the rats body weights caught up to the pair fed group . Next, energy expenditure could have been confirmed using whole body calorimetry. The gene analysis using RT-PCR could have included other tissues such as adipose tissue and liver for UCP2 analysis. Also other genes responsible for fat metabloism (fatty acid synthase and lipase) and skeletal muscle catabloism (ubiquitin pathway) could have been measured, and shed light on how the rats lost weight as both fat and skeletal muscle. Lastly, in order to publish the study, the analysis mentioned in the limitations of the study and further gene analysis needs to be conducted to confirm the genes responsible for the increased energy expenditure and also the fat and skeletal muscle catabolism that caused the weigh loss in the rats.

Chapter 5

Prevention of Diet Induced Obesity in C57BL/BJ Mice with the Addition of Green Tea but not with Cocoa or Coffee to a High Fat Diet

5.1 Introduction

The study detailed in Chapter 4 investigated how the infusion of SAR-Ang II induced CHF and associated wasting. It also attempted to address the origin of elevated energy expenditure in cardiac cachexia. The possible causes for both cachexia and obesity may arise from the same genetic regulation of key energy metabolism proteins (e.g., UCP2 and UCP3) (Dulloo & Samec, 2001). From this perspective, the study of body growth, composition and energy expenditure in obesity may also shed light on mechanisms that are also involved in RVP or SAR-Ang II induced cardiac cachexia.

Obesity is widely accepted to be the imbalance of energy intake and energy expenditure, which influences the development of insulin resistance (Murase, et al., 2002). Obesity is also a factor contributing to cardiovascular risk profile and significant mortality (Wolfram, et al., 2005). Previous research suggests that the risks of developing obesity are reduced by feeding green tea catechins in the diet (Murase, et al., 2002). The thrifty phenotype hypothesis proposes that poor nutrition early in life results in development of type 2 diabetes and the metabolic syndrome which are associated with obesity (Hales & Barker, 2001). Thus, feeding correct amounts of polyphenol compounds in the diet may affect the development of disease later in life or reduce its probability of development.

The development of obesity using a long-term animal model of high fat feeding in mice is quite common, particularly using the CJBL57 mouse strain (Surwit, et al., 1995). The pathogenesis of obesity in this mouse model is associated with increased weight and adipose tissue as a percentage of carcass weight. The intracellular triglyceride content is increased in skeletal muscle during insulin-resistant states (e.g., obesity or high-fat feeding in mice). Decreased lipolysis is currently implicated as a key mechanism involved in the development of obesity (Kim, et al., 2003). Consumption of green tea is known to increase lipolysis.

Green tea is a commonly consumed beverage worldwide (i.e., global consumption of 0.12L/ annum/ person), with a multitude of health benefits from its consumption ranging from its rich polyphenol (i.e., catechin) content to the relaxing effects (i.e., theanine) (Graham, 1992). There is rapidly growing evidence supporting the use of anti – oxidants and related compounds to prevent or to be used as therapies to treat diet-induced obesity (i.e., Asteraceae, Green Tea, EGCG. (Abid, et al., 2007; Han, et al., 1999). Polyphenols can be administered to CHF cachexia patients as an oral supplement in hypertensive humans and animals (Gompf, 2005). On the basis of a wide number of animal and human studies, green tea catechins have been shown to possess properties which inhibit deposition of body fat and weight gain leading to obesity, via decreased energy absorption and increased lipolysis and fat oxidation (Klaus, et al., 2005; Murase, et al., 2002; Murase, et al., 2006). Typically green tea has been delivered as a beverage in the fluid intake of the diet.

To date, there have been few studies investigating the effects of adding whole food substances containing polyphenols (e.g., green tea, cocoa powder) directly to food and observing their effects on the development of diet-induced obesity. Some studies have extracted green tea catechins from green tea powder and added them to food (Murase, et al., 2002; Raneva, et al., 2005) to stimulate body weight loss. However, in the present study the inclusion of the whole green tea to the diet of the animals to replace some of the carbohydrate component makes this particular study quite novel.

The present study seeks to test whether the inclusion of cocoa, coffee or green tea; which all contains polyphenols and caffeine, will provide protection from DIO. It has been observed that the type of dietary fats used in the diet can dictate the degree of DIO (i.e., different rates of lipid metabolism and intestinal absorption) (Mori, et al., 2007). Thus butter fat was chosen as it previously has been used to induce DIO (Darlene et al., 2006). In consideration of the altered glucose and fat metabolism that occurs in obesity, this study will also explore the possible interactions between plasma metabolites and the development or suppression of body weight gain and increased adipose tissue in the carcass composition.

The principle hypotheses tested in this study is that the feeding of green tea in a high fat diet results in development of an obesity protective physiology. This may be the result of elevated lipolysis (catechin induced) and use of these liberates fatty acids as fuel for oxidation attributable to increased energy expenditure. Further, organs (i.e., liver), may change in weight due to release of hepatic triacylglycerides and thus lead to reduced liver weight (Bose, et al., 2008). The effect of feeding different polyphenol containing foods in adult mice was observed by measure of body weight, and its composition for a 16-week period.

5.2 Materials and Methods

5.2.1 Animals

The animals were the C57BL/6J mice (obesity prone). They have been compared with other mouse models of obesity to characterise their phenotype. There were four groups of n = 12/ group.

5.2.2 Diets

There were four diets composed. The standard diet containing 21% fat, 19% protein, 49% carbohydrate, 0.15% cholesterol, and a semi-pure rodent diet (Specialty Feeds, WA, Australia). The other three diets consisted of the standard diet with the exception that they contained 2% of the following, Bourneville cocoa (Cadbury, TAS, Australia), coffee (Vittoria, NSW, Australia), and green tea (Good Young, Taipei, Taiwan). For a more detailed outline of dietary composition, see Table 5.1 below.

Table 5.1 Composition of Feed

Ingredient	Feed Composition	Ingredient	Feed Composition
Sucrose	34.1% (36.1% in control mice feed)	*Butter Fat*	21%
Beverage	2% (w/w) (0% in control mice feed)	*Methionine*	0.3%
Wheat Starch	15%	*Cholesterol*	0.15%
Cellulose	5%	*TD88137 Minerals*	0.14%
Casein (Acid)	19.5%	*TD88137 Vitamins*	1%
Ca^{+} Carbonate	1.7%	*Antioxidants*	0.004%

5.2.3 Housing

Mice were housed in Perspex boxes (0.7m x 0.25m x 0.25m), with sawdust bedding and one tissue added for nest building. Bedding was changed every two weeks or when the bedding became soiled.

5.2.4 Food intake, fluid intake and live weights

Live-bodyweight was recorded on a weekly basis (see Figure 6.1), and food/ water intake on a thrice weekly basis (see Figures 6.2 and 6.3). The balance used was a AND GX-4000. A six measurement averaging calculation was used when determining live-bodyweight.

5.2.5 Faecal collection

Faeces were collected at week 8 for determination of energy digestibility. Paper was lined on the bottom of the mouse box and a sieve was used to filter the faeces from the paper bedding. The faeces was dried at 85^{o}C to a constant weight, to determine dried weight and then ground for measurement of gross energy in a bomb calorimeter (PARR, Illinois USA).

5.3 Analytical Methods

5.3.1 Glucose tolerance test

The mice were fasted for 8 hours overnight yet still had access to *Ad libitum* H_2O. The mice were firstly restrained and a small knick (cut) was made at the terminus of the tail and a blood sample (5µl) was taken for measurement in a cuvette and placed in a Hemocue analyser (Ängelholm; SWEDEN) for plasma glucose determination**.** After the time 0, a blood sample was taken, (as described above) by slowly massaging a drop of blood from the tail vein and plasma glucose measured using the Hemocue analyser. A bolus of L-dextrose (20% w/v) was administered to the mouse and the volume determined according to the mouse live body weight – see equation below;

Volume of glucose needed for bolus (µl) = [(Mouse body weight (g)) / 40] x 200

Dosage = 1g per kg body weight

After this point, blood samples were taken in the same fashion as previously mentioned at t = 30, 60, 90, 120 and 180mins post bolus loading. The points were then plotted on a graph; the area and the concentration of the plasma glucose were measured. The area under the curve was determined using Cricket Graph Software (Version 1.0.5)

5.3.2 Dual X-ray Absorptiometry

DEXA measurements were performed with the Norland pDEXA Sabre (Fort Atkinson, U.S.A.) and the Sabre Research software (pDEXA calibration version 3.9.4). Prior to testing, the QA (Quality Assurance) and QC (Quality Control) were run to calibrate the machine. Initially, anaesthesia was delivered intraperitoneal (60mg/kg, 9mg/kg) comprising of ketamine/ xylazine (~0.1mL). When the mice lost consciousness they were placed on their ventral side and their limbs were taped to the pDEXA Sabre. The mice were analysed for fat, fat-free, bone mass and results tabulated as mass (g) and as a percentage of live body weight. The data collected were; BMD (Bone Mineral Density – cm^2), BMC (Bone Mineral Content – g), lean (g) and fat (g).

5.3.3 Anaesthesia

The anaesthesia administered for termination of the mice was Nembutal (200mg/kg dosage). For anaesthesia used during DEXA please see Chapter 5.3.2.

5.3.4 Blood and tissue collection

After the mouse was placed under anaesthesia (for dose and volume refer to Chapter 5.3.3) it was tested for reflex (eye, tail pinch). When unconscious, a lateral incision was made up the abdominal cavity and the liver, kidney, tail length, and epididymal fat were removed and weighed.

5.3.5 Statistical Analysis

Statistical analysis was conducted using SPSS software (V.12) using a 1 – way ANOVA at a $p<0.05$ and 0.01. When an observation was made over a period of time, a repeated measures ANOVA was performed at a $p <0.05$ and 0.01.

5.3 Results

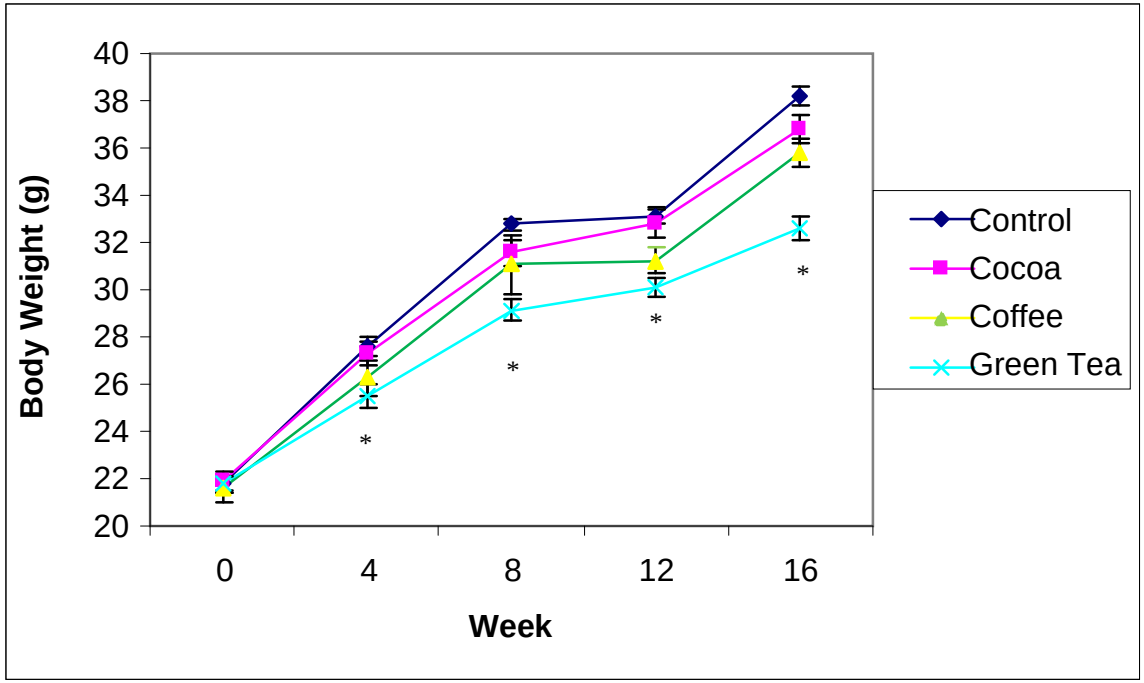


Figure 5.1 Progressive live-body weight (g) over an 16 week period for control, cocoa, coffee, and green tea groups. Data displayed as mean ± standard error. Control vs. treatment * $p<0.05$

As displayed in Figure 5.1, all groups gained weight over the 16 week period. However, from week 3, the green tea (2%) group displayed lower ($p<0.05$) body weight gain when compared to the control group. There was no difference in feed intake between groups over the 16 week period (Figure 5.2) or fluid intake (Figure 5.3). As displayed in figure 5.4; the green tea group had increased ($p<0.05$) BMD and in Figure 5.5 there is evidence of lower ($p<0.05$) BMC than the control group.

There was decreased ($p<0.05$) body fat (g) in the coffee group and green tea group ($p<0.01$), with the green tea group also displaying decreased ($p<0.01$) total body weight (g) and increased lean tissue ($p<0.05$), as seen in Figure 5.6. As shown in Figure 5.7, the green tea group displayed increased ($p<0.01$) lean and decreased ($p<0.01$) fat as a percentage of total body weight.

As observed in Figure 5.8, the coffee and green tea groups displayed lower ($p<0.05$) BMC in the hind limb yet only the green tea group displayed lower abdominal BMC ($p<0.01$) as well. In Figure 5.9, the green tea group displayed elevated ($p<0.01$) lean tissue and decreased ($p<0.01$) adipose tissue as a percentage of both hind limb and abdominal weight. There was no change in glucose area under the curve in any of the groups (Figure 5.11).

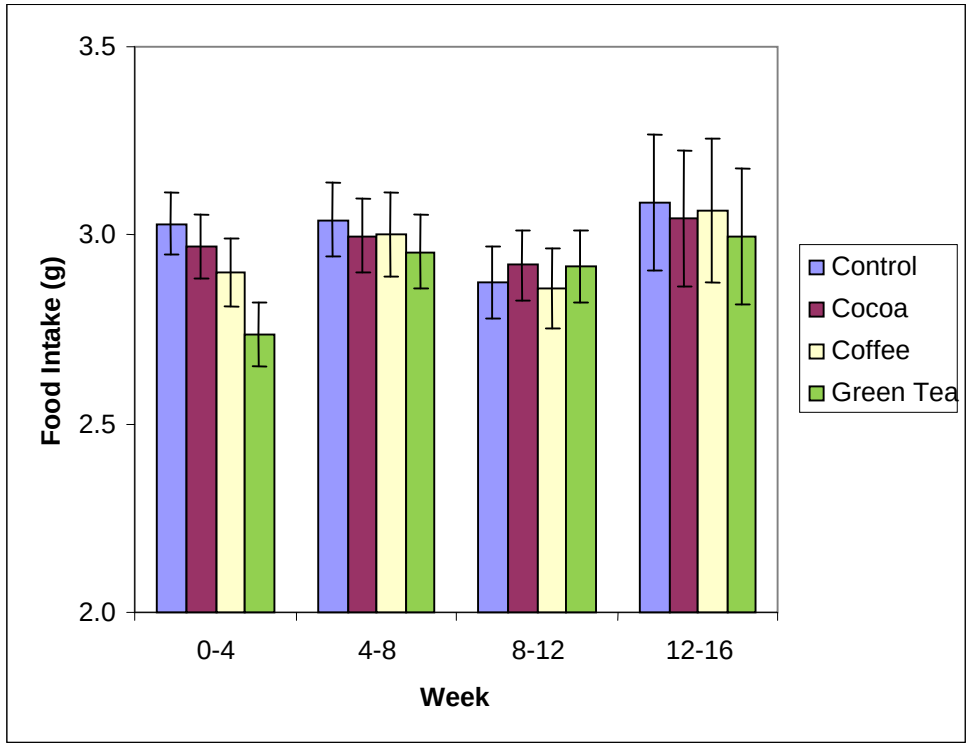


Figure 5.2 Food intake (g) over a 16 week period for control, cocoa, coffee, and green tea. Data displayed as mean ± standard error. Control vs. treatment $^{*}p<0.05$

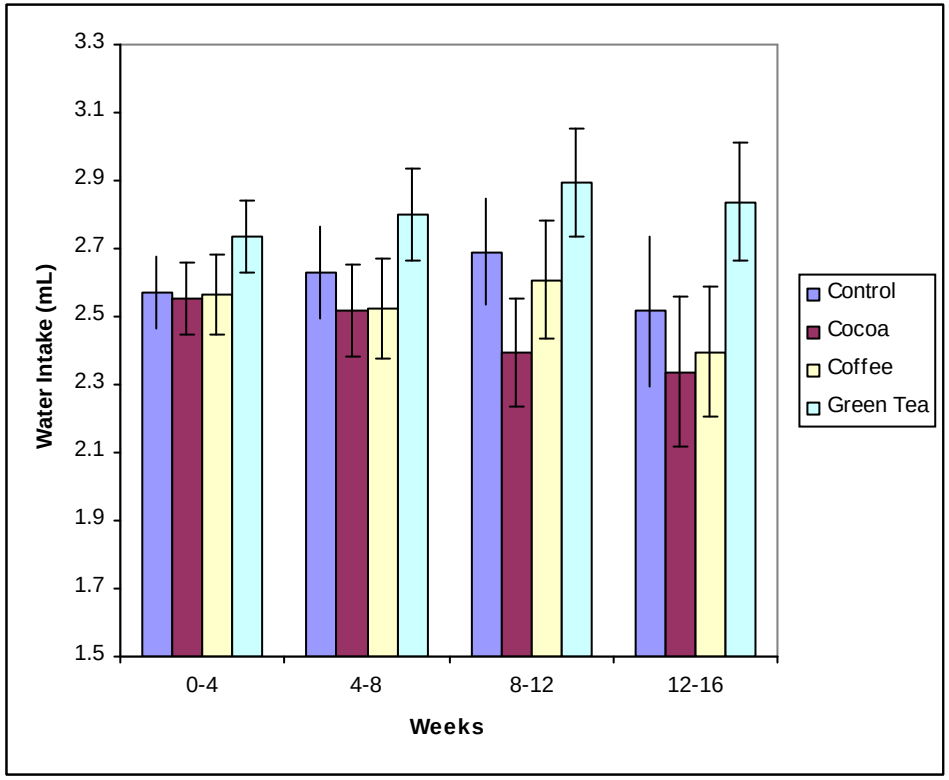


Figure 5.3 Water intake (mL) over a 16 week period for control, cocoa, coffee, and green tea groups. Data displayed as mean ± standard error (number of observations).

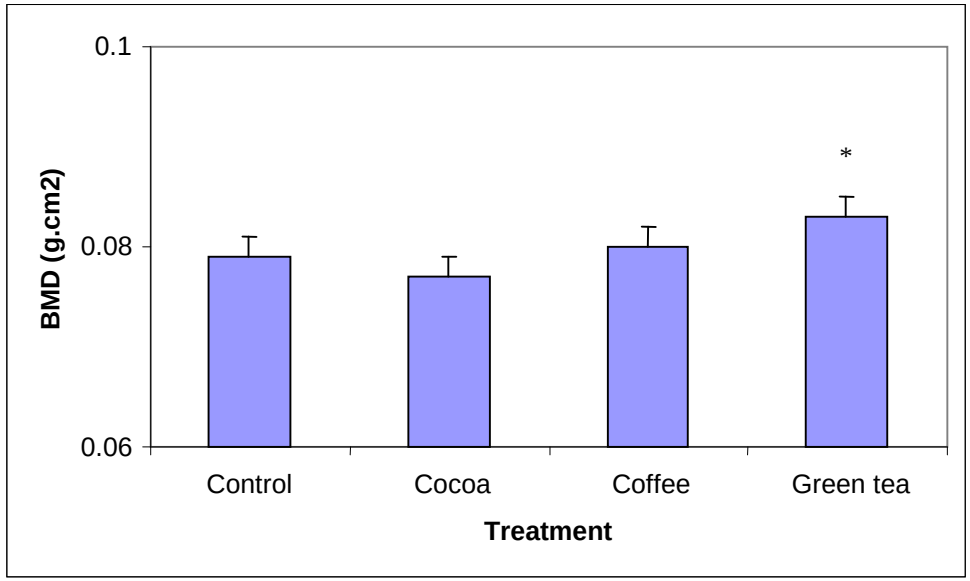


Figure 5.4 Bone Mineral Density (BMD) for control, cocoa, coffee, and green tea. Data are expressed as either per gram basis or as a percentage of total body weight. Data are displayed as mean ± standard error. Control vs. treatment * $p<0.05$.

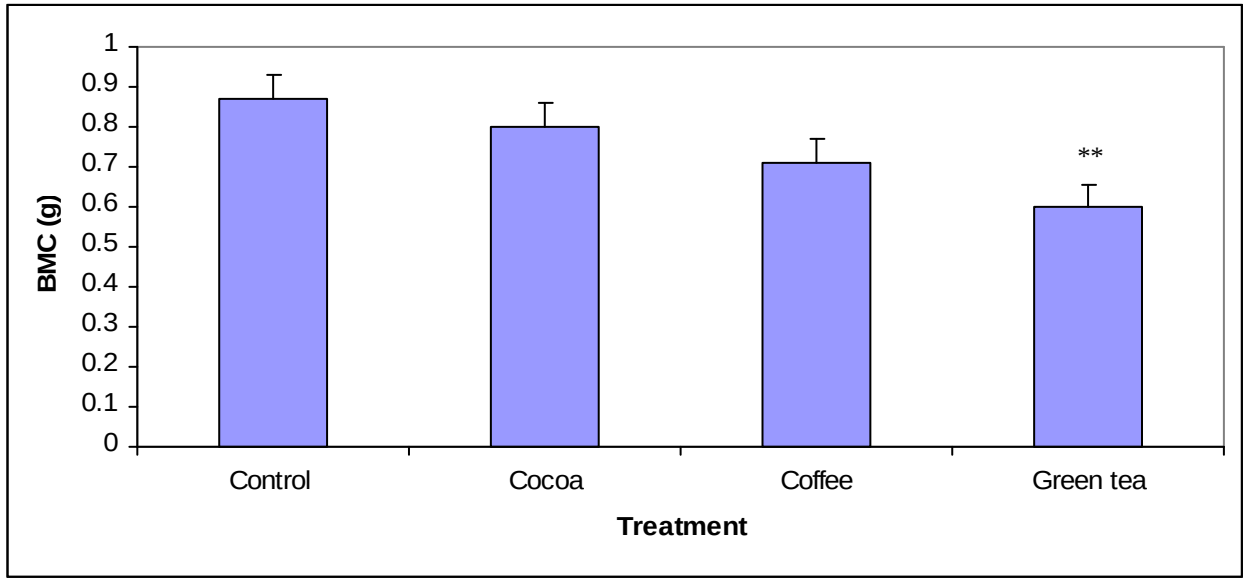


Figure 5.5 Bone Mineral Content (BMC) per (g). Data are displayed as mean ± standard error. Control vs. treatment * $p<0.05$, ** $p<0.01$

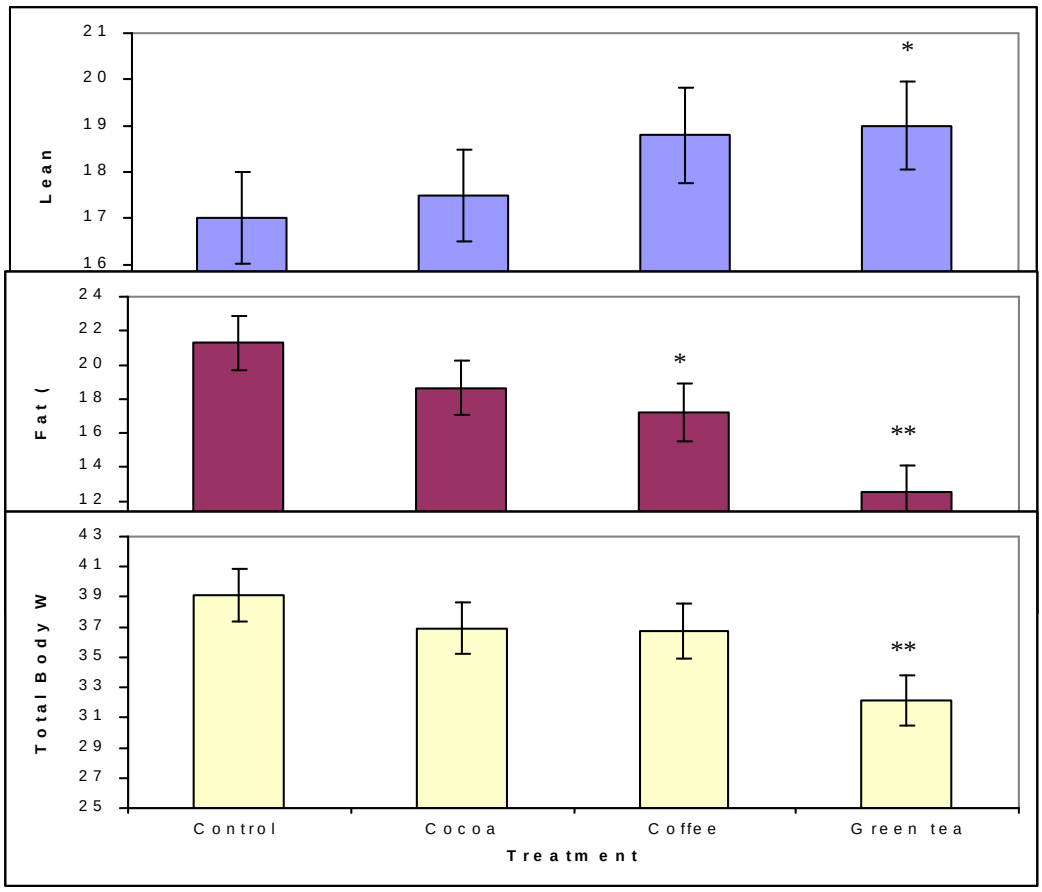


Figure 5.6 Body composition components per (g). Data are displayed as mean ± standard error. Control vs. treatment * $p<0.05$, ** $p<0.01$

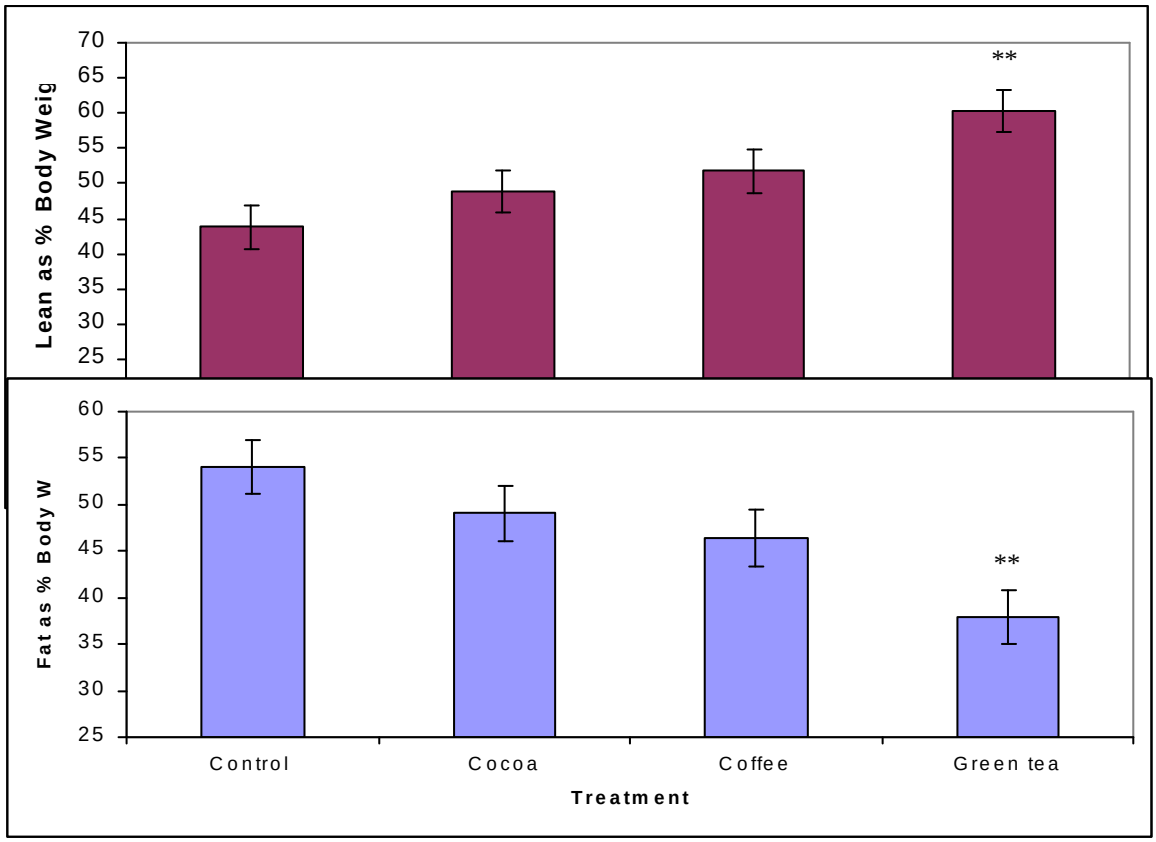


Figure 5.7 Body compositional components as a percentage total body weight. Data are displayed as mean ± standard error. Control vs. treatment * $p<0.05$, ** $p<0.01$.

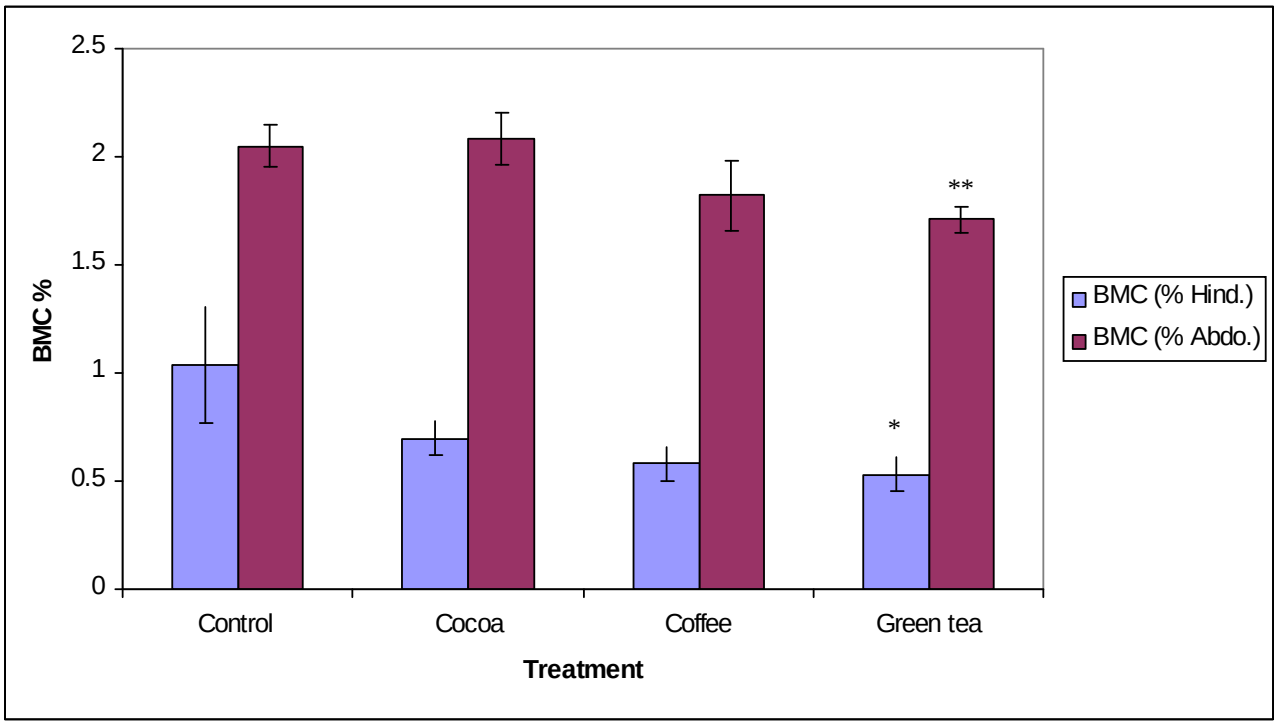


Figure 5.8 Bone Mineral Content at Week 16 for control, cocoa, coffee, and green tea groups. (As a percentage of Hind limb and Abdominal sections). Data are expressed as either per percentage of hind limb or abdominal weight and displayed as mean ± standard error. Control vs. treatment * $p<0.05$, ** $p<0.01$

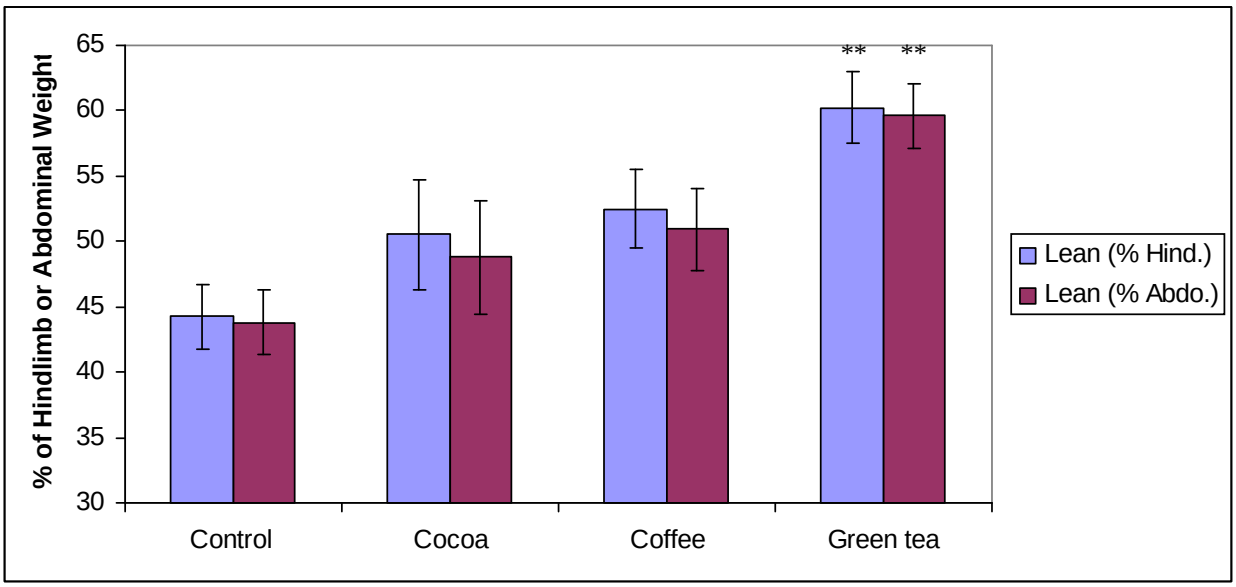


Figure 5.9 Lean as a percentage of either hind limb (% Hind.) or abdominal (% Abdo.) region for control, cocoa, coffee, and green tea groups. Data are expressed as either per percentage of hind limb or abdominal weight. Data are displayed as mean ± standard error. Control vs. treatment * $p<0.05$, ** $p<0.01$

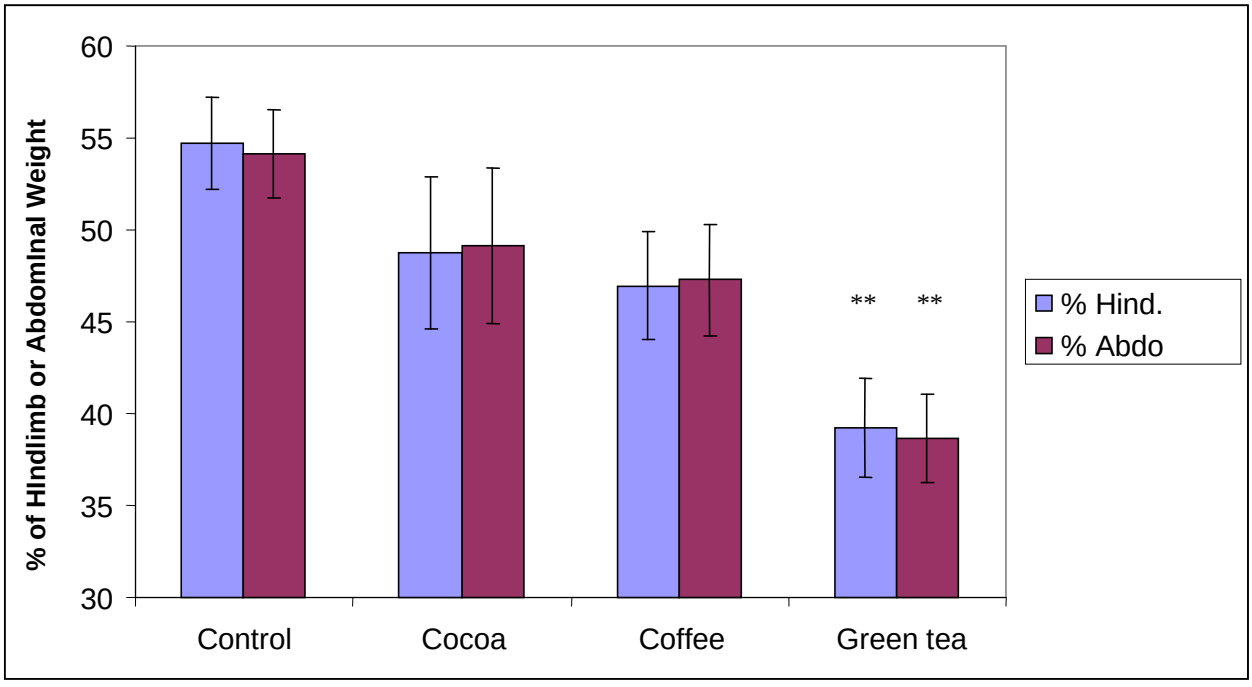


Figure 5.10 Adipose tissue (fat) as a percentage of either hind limb (% Hind.) or abdominal (% Abdo.) region for control, cocoa, coffee, and green tea groups. Data are expressed as either per percentage of hind limb or abdominal weight, and as mean ± standard error. Control vs. treatment * $p<0.05$, ** $p<0.01$

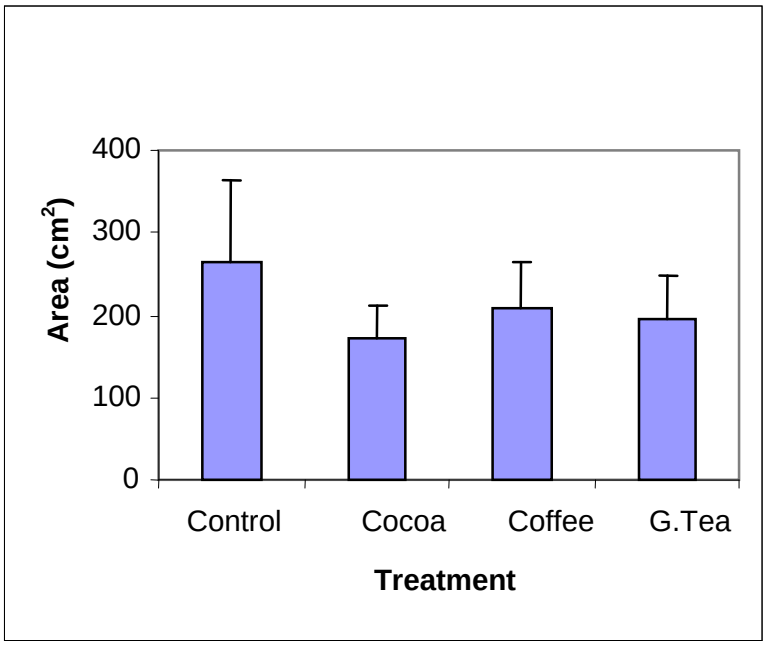


Figure 5.11 Glucose Tolerance Test - area under curve (cm^2). Data are presented as mean ± standard error. Control vs. treatment * $p<0.05$

Table 5.2 Liver, Kidney and Epididymal Adipose Tissue as a percentage of body weight. Data is expressed as either per gram basis or as a percentage of total body weight. Data is displayed as mean ± standard error (number of observations). N.B. EWAT (Epididymal white adipose tissue), Control vs. treatment $^{*}p<0.05$, $^{**}p<0.01$

Section	Control	Cocoa (2%)	Coffee (2%)	Green Tea (2%)
Liver (% L.B.W.)	6.66±0.54 (12)	6.82±0.47 (12)	6.72±0.40 (10)	5.77±0.24 (12)**
Kidney (% L.B.W.)	1.02±0.03 (12)	1.06±0.04 (12)	1.23±0.07 (10)*	1.08±0.05 (12)
EWAT (% L.B.W.)	5.83±0.42 (12)	5.57±0.47 (12)	5.49±0.37 (10)	5.84±0.54 (12)

As seen in Table 5.2, the green tea group displayed lower ($p<0.01$) liver mass as a percentage of body weight. In addition, the kidney was greater ($p<0.05$) in the coffee group as a percentage of body weight. The green tea group displayed higher ($p<0.01$) plasma NEFA concentrations (Figure 5.12), whilst there are no changes in the cocoa or coffee group. There were reduced ($p<0.05$) plasma glucose concentrations (mM) (Figure 5.13) in the cocoa group (14.8%) as compared with the control group.

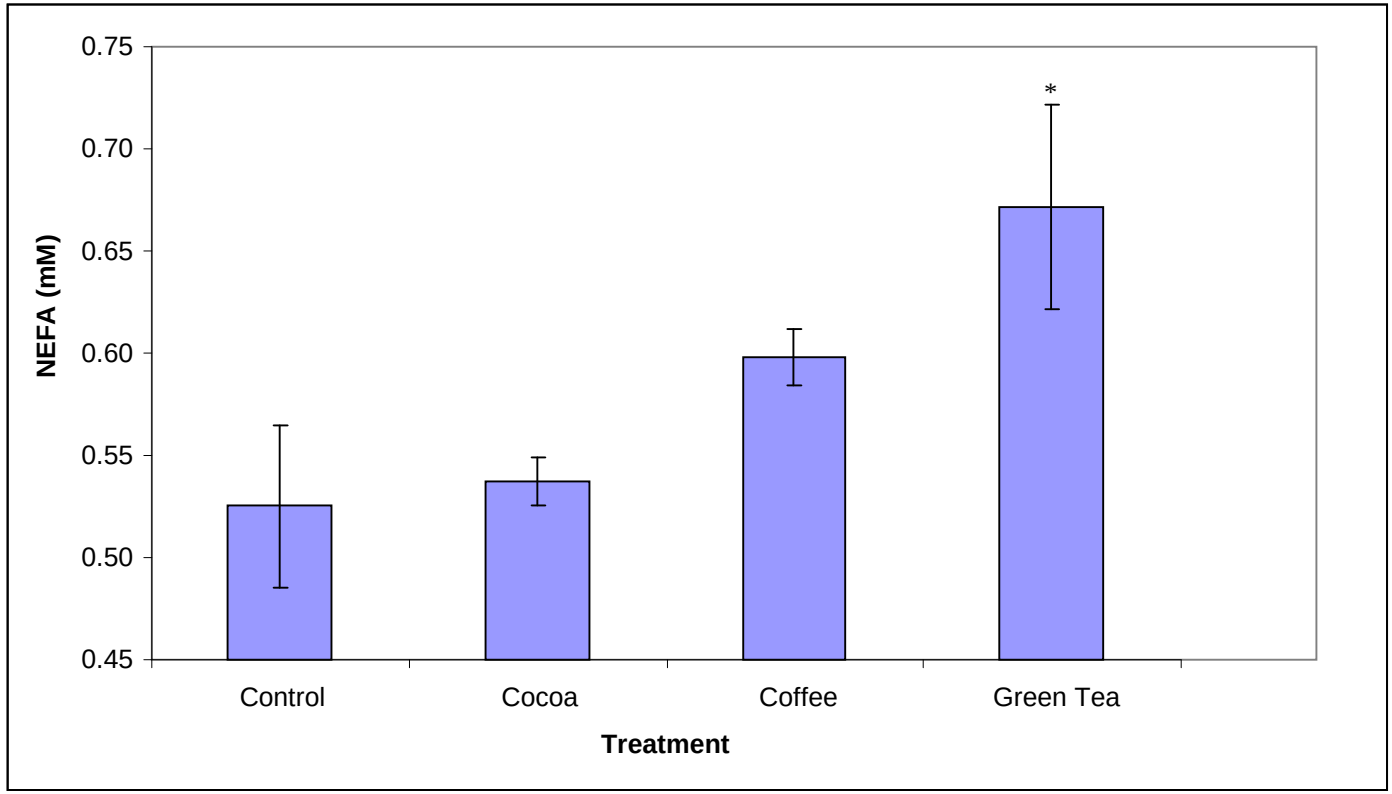


Fig 5.12 Plasma NEFA levels. Data presented as mean ± standard error. Control vs. treatment * $p<0.05$

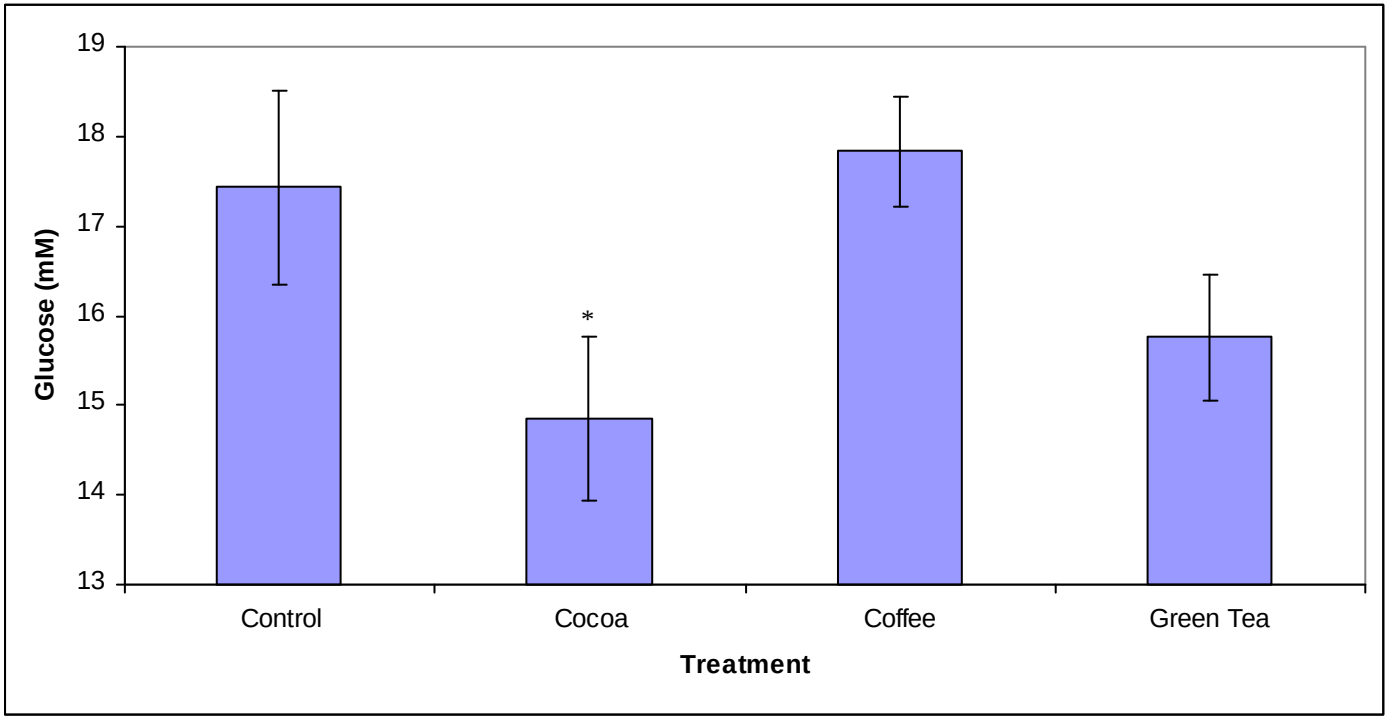


Figure 5.13 Terminal Plasma Glucose. Data presented as mean ± standard error. Control vs. treatment * $p<0.05$

5.4 Discussion

It was proposed in the present study that feeding green tea in the diet would impede the on-set of DIO (Diet Induced Obesity). The present study showed for the first time *in vivo* that feeding green tea in a model of reduces DIO body weight gain, promotes skeletal muscle deposition and reduces adipose tissue content. The obesity-protective actions of green tea in beverage format without consumption of a high fat diet has been shown by (Han, et al., 1999), and (Wolfram, et al., 2005).

The proposed mechanism may be a metabolic effect by a compound in green tea which decreases energy for fat storage (i.e., increased energy expenditure) and promotes lower body weight gain. Supporting this proposal, it has been suggested that the weight loss from consuming green tea is due to elevation of metabolic rate (Komatsu, et al., 2003). It may be that green tea catechins possess fat oxidation properties, particularly the green tea polymerized polyphenols as seen by (Rumpler, et al., 2001) in oolong tea or (Dulloo, et al., 1999) in green tea, rather than using caffeine alone (i.e., coffee group).

It was proposed that the weight inhibition by green tea would be mainly due to decreased fat deposition. The feeding of green tea with a high fat diet produced a significant decrease in adipose tissue. (Ashida, et al., 2004) observed that green tea consumption leads to reduced adipose tissue deposits without change in body weight, skeletal muscle content, and food or water intake. The feeding of green tea catechins to induce weight loss has been documented in a number of studies (Chen, et al., 1997; Kao, et al., 2000). Kao et al., (2000) demonstrated that intra-peritoneal administration of EGCG induces weight loss. (Chen, et al., 1997) showed that after oral administration of EGCG very little is absorbed (1.6% of administered amount), and

thus feeding raw green tea powder versus drinking green tea may possess a digestive protective mechanism for EGCG absorption.

The results of the green tea group in this study are a direct contrast to the cocoa group and a previous study by Matsui, et al., (2005), who showed that cocoa inhibits high-fat diet-induced obesity, by suppressing hepatic fatty acid synthase and up regulating UCP-2 (thermogenesis factor). Moreover, in the present study only 2% cocoa (by weight) was added to the diet as opposed to 12% by (Matsui, et al., 2005), thus a differing dose weight. However, supporting the proposed hypothesis that feeding cocoa with a high fat diet does not reduce body weight or adipose tissue, findings from this study agree with findings by (Hasegawa, et al., 1991) (e.g., mice overeating cookie and chocolate mashed diet developed mild hyperglycaemia, with relative obesity from 16 weeks of age).

The levels of caffeine and polyphenols in the diets were not analysed and there is no information regarding dosage. Thus, there is no room to speculate about the effects of altering dietary quantities of these compounds and how this variation would affect corresponding adipose tissue or weight loss. To make any further conclusion, accepted and published nutritional values may be used. However, if one considers that the cocoa, coffee, and green tea diets all contain caffeine at a constant level it is possible to suggest that the differences in body weight and composition are due to the presence of green tea catechins. The presence of caffeine and non-caffeine compounds (i.e., chlorogenic acid and quinides) in the coffee diet was not sufficient to inhibit diet-induced obesity, and did not alter glucose metabolism, contradicting findings by (Greenberg, et al., 2005). However, the present study documented some weight loss due to fat loss. In agreement with this observation, (Zheng, et al., 2004) observed that feeding caffeine reduced intraperitoneal adipose tissues and body

weight and showed that catechins and caffeine work synergistically to prevent obesity. It is possible that reduced body fat (g) in the coffee group mice may have been due solely to the actions of caffeine via increased lipolysis in rat epididymal adipose tissue, epididymal fat pad weight and reduction of mean adipocyte diameter as documented by (Cheung, et al., 1988), in rats. Thus caffeine alone is a highly relevant compound when considering increased rates of lipolysis and energy expenditure for weight loss in obesity.

It was assumed in the present study, that any weight loss would be due to increased energy expenditure and not decreased feed intake. In the present study, feeding of green tea did not alter intake of the composite diet. Indeed, the finding of unaltered feed intake agrees with numerous studies (Yang, et al., 2001) (i.e., oolong tea), (Raneva, 2005) (i.e., green tea catechins) and (Han, et al., 1999), (i.e., oolong tea powder (5% of diet)). The weight loss and change in body composition in the green tea group may have been caused by an increase in metabolic rate (i.e., UCP's). Similarly, (Hasegawa, et al., 2003) observed that body weight is suppressed whilst food intake is unaffected during green tea consumption. There was no decrease in body weight in the coffee group, or feed intake. This may have been due to increased appetite and thus feed intake. Interestingly coffee odorants have been found to stimulate appetite (Dorri, et al., 2007) and thus may have increased feed intake and thus body weight. These results show that feeding a high fat diet with the inclusion of green tea, but not cocoa or coffee, decreased body weight and fat. The proposed mechanism for this lipid specific weight loss is caffeine and catechin's influence on lipolysis.

Notably, changes in body weight may have had a direct and proportionate effect on BMD. It can be hypothesized that the absence of a high fat diet and the

presence of obesity, the BMD of the control mice may have been lower as per the BMD of the green tea group mice. Green tea directly increased BMD agreeing with (Devine, et al., 2007; Muraki, et al., 2007; Shen, et al., 2008). These findings suggest that changes in fat and protein metabolism in mice fed a high fat diet with green tea may influence bone development. Several studies have suggested that BMD is negatively influenced by coffee consumption and oxidative stress, and that the intake of green tea, green tea polyphenols and caffeine can have a positive influence on BMD development (Devine, et al., 2007; Grassi, et al., 2007; Hegarty, et al., 2000; Muraki, et al., 2007; Shen, et al., 2008).

It was assumed that cocoa and coffee would have no effect on BMD. The present study found that there was no change in BMD in the cocoa or coffee group. As mentioned in the previous paragraph, this unchanged BMD in comparision to the control animals may reflect higher body weight and body fat. These results support other studies that show that cocoa flavoured beverage products have been shown to have no effect on bone density, growth, or size (Gibbons, et al., 2004), and that coffee (i.e., caffeine) may actually induce higher rates of spinal bone loss (>300 mg/d) (Harris & Dawson-Hughes, 1994). In contrast, (Conlisk & Galuska, 2000) found that caffeine intake was not a significant predictor of BMD after adjusting with linear regression models for potential confounders (i.e., height, BMI, age, calcium and protein intake, alcohol and tobacco use), nor was it a signifant predictor of BMC (Wetmore, et al., 2008). In comparision with BMD, green tea mice displayed lower BMC. These results agree with findings by (Toth, et al., 2005), who observed a positive association between femur neck bone mass and decreased BMD, and lower body weight, linked with repetition of diet (Bacon, et al., 2004) .

In this context of decreased body weight, BMD and BMC in the green tea group also was aligned with decreased fat (g) and decreased as a percentage of carcass. Green tea's lipolytic effects are well demonstrated, decreasing hepatic fat content, and elevating hepatic β–oxidation (Murase, et al., 2005; Wolfram, et al., 2005), being possibly due to fatty acid synthesis inhibition (Dulloo, et al., 2000; Tian, et al., 2004). A proposed mechanism to explain the effect of green tea to protect against obesity development may be due to decreased visceral fat deposition (Nagao, et al., 2007), elevated thermogenesis and fat oxidation (Dulloo, et al., 2000; Murase, et al., 2005) and being partially due to increased faecal lipid and cholesterol excretion causing decreased fat absorption (Hsu, et al., 2006; Yang, et al., 2000). However, (Ashida, et al., 2004) suggested that green tea reduces adipose tissue weight without any changes in body weight, skeletal muscle weight. Further, feed and water intakes were not altered meaning there was a lypolytic action causing adipose tissue weight.

In the present study, feeding of 2% cocoa with a high fat diet did not inhibit adipose tissue development. This result contrasts findings by (Matsui, et al., 2005). They proposed a mechanism for fat loss in cocoa fed mice to be due to decreased mesenteric white adipose tissue weights via increased energy expenditure (i.e., increased hepatic UCP2 mRNA expression). Certainly the difference in actual cocoa used to formulate the diet (12.5% vs. 2%) and thus the assumed lower polyphenol content of the present studies cocoa based diet would have caused this difference in findings.

It has been suggested that chlorogenic acid (present in coffee) reduced glucose absorption by 6.9%, suggesting that long term use of coffee particularly rich in chlorogenic acid may reduce body mass and body fat (Thom, 2007), possibly explaining reduced body fat in the coffee group. However, feeding 2% coffee with a

high fat diet does not inhibit adipose tissue development. Supporting this hypothesis, (Lopez-Garcia, et al., 2006) observed that coffee had no effect in changing fat or lean tissue carcass composition as it had a small reduction in long-term weight gain. Consistent with this hypothesis, plasma NEFA were also unchanged in the coffee group.

In a series of studies, it was suggested that cocoa in combination with fructo-oligosaccharides actually improves calcium absorption (Fukushima, et al., 2006). However, the presence of cocoa and chocolate (+ methyloxantine theobromine), fed to mother rabbits causes decreased bone development in progeny and incomplete ossified bones (Skopinski, et al., 2003; Tarka, et al., 1986). Consistent with this observation, there was no body weight loss effect of cocoa incorporation into a high fat diet and any change in BMD or BMC in mice.

In parallel with bone development, increased body weight and fat for the cocoa and coffee groups, contrast with findings by (Matsui, et al., 2005) and (Shimoda, et al., 2006). Of a particular note is that both these groups of authors used higher rates of cocoa (12% versus 2%) and coffee (1% green coffee bean – unroasted) respectively than the present study. On this basis it is possible to suggest that higher amounts of anti-oxidants containing beverages fed to mice would have most certainly influenced body composition outcomes, even though the amount fed may be unrealistic or extremely bitter in taste.

Prior to investigation, it was hypothesized that feeding green tea would impede obesity development largely through decreased visceral adipose tissue. In contrast to the present study, (Wolfram, et al., 2005) observed that a diet rich in EGCG (1%) in combination with a high fat diet (20% w/w fat and 26% w/w sucrose) significantly decreased epididymal adipose tissue. A possible explanation for the

unchanged epididymal adipose tissue in the green tea group, is that there was a higher levels of sucrose in the diets of mice in the present study (36%) which may have altered fat distribution and storage in animals.

Numerous epidemiological studies indicate that regular coffee consumption reduces the risk of developing type 2 diabetes. However, decaffeinated coffee proves more effective in combating insulin resistance (Shearer, et al., 2007), possibly via delayed glucose absorption (chlorogenic acid content) (Thom, 2007). Furthermore the findings of the present study where the mice in the coffee group displayed reduced ($p<0.05$) glucose at t = 120mins agree with coffee's ability to reduce the risk of type 2 diabetes development. However, it must be noted that there was no other significant changes in respect to the GTT area under the curve.

Studies investigating the effects of green tea consumption show that liver weight decreases via decreasing hepatic lipid deposition/ triglycerides as EGCG acts to reduce hepatic weight, fat content and increase hepatic energy status (Bose, et al., 2008; Fiorini, et al., 2005; Hasegawa, et al., 2003). In agreement, the present study showed that feeding green tea with a high fat diet reduces liver weight. To further explain possible mechanisms causing this liver weight change, UCP's are known to increase hepatic β-oxidation with feeding of green tea (Murase, et al., 2002), while (Dulloo, et al., 1999) observed tea catechin ingestion stimulates O_2 consumption and energy expenditure. Thus, elevated hepatic energy expenditure through UCP thermogenic effect and utilisation of hepatic fatty acid stores may have contributed to decreased liver weight.

When compared with the kidney mass as a percentage of live body weight, the coffee group displayed a significantly greater mass at $p<0.05$ (+17% versus control group), than all other groups. (Curhan, 1996) proposed that kidney stones may be

responsible for higher kidney weights in patients with a higher consumption of caffeinated coffee (+ 10% of normal intake) whereas (Ha, 1998) proposed kidney hypertrophy being possibly caused by increased blood pressure. In the present study, higher kidney mass was one consequence of higher coffee intake. This finding may suggest that coffee intake may exacerbate higher blood pressure with morbid obesity.

Feeding green tea caused a significant elevation of plasma FFA. A recent study shows that feeding green tea elevates plasma FFA/ NEFA (Murase, et al., 2005), through increased lipolysis as oolong tea has been traditionally known to have anti-obesity and hypolipidaemic effects (Han, et al., 1999), and initiates inhibition of lipogenesis in adipose tissue (Hasegawa, et al., 2003). This change may be mediated by glycerol release from fat cells (Mochizuki & Hasegawa, 2004). Thus, in the present study, this explains why the GTT was unaffected in the green tea group, as NEFA may have been the chief source of calorie use and thus glucose was not preferentially utilized. However, in contrast, several studies showed a tri-acyl glyceride lowering effect of green tea (Ashida, et al., 2004; Serisier, et al., 2008) and increased GLUT-4 activity. Further work is needed to quantitify the activity of GLUT-4 and other glucose metabolism enzymes before speculating further.

In this context, it is possible to suggest that the action of green tea may have also been attributed to the synergistic effect of catechins in green tea. Their summative relationship has been well documented by a number of investigators (Han, et al., 1999; Kobayashi-Hattori, et al., 2005). One could speculate that the action of green tea catechins and caffeine is due to a metabolic sink (e.g., elevated energy expenditure), and that increased NEFA and decreased body fat are physical manifestations of this consequence of elevated energy expenditure. In support of this hypothesis, (Arciero, et al., 1995) postulated that the effect of caffeine may be

achieved through elevation of metabolic rate and fatty acid availability via lipolysis of adipocytes and consequent release of catecholamines. On this basis, the present model could be re-eveluated to investigate the activity of uncoupling proteins, and thus yield the answer to the high rate of lipolysis as evidenced in the green tea group.

However, despite the effect in the green tea group, there was no change in either the cocoa or coffee groups in respect to plasma NEFA despite the assumed presence of caffeine. In one study, there was an observation showing that in conjunction with exercise, coffee consumption actually reduces lipolytic rate by suppression of lipolysis (Mougios, et al., 2003). Yet in habitual coffee drinkers significantly elevated plasma FFA prevails (Cocchi, et al., 1983; Denaro, et al., 1991) indicating an increase in lipolysis. The results of the present study demonstrate that lipolysis is attributed to the combined effect of green tea catechins and caffeine in the green tea or solely green tea catechins. Cocoa does not affect NEFA plasma concentration unlike (Matsui, et al., 2005), nor does coffee in the context of this dietary model in accordance with (Mougios, et al., 2003).

This study has found that mice did display decreased plasma glucose in the cocoa group which agrees with (Tomaru, et al., 2007) who observed that dietary supplementation with cacao extract containing proanthocyanidins inhibited hyperglycaemia in diabetic obese mice. However, the unchanged glucose concentration in the green tea group contradicted findings by (Miura, 2005), who observed green tea to have hypoglycaemic properties in type II diabetic mice. EGCE the major active catechin found in green tea acts synergistically with caffeine to induce thermogenesis and inhibits α-amylase and sucrase activity in rat intestine, ultimately delaying glucose uptake from rat intestine (Wolfram, et al., 2005).

In this context, the present study has identified that feeding of a readily available and inexpensive food ingredient (e.g., 2% green tea) fed to animals on a high fat diet presents improved health parameters for the morbidity obese or individuals with high risk of obesity development and who currently consume a high fat diet. To gain a broader insight into the underlying physiological mechanisms which may be responsible for the inhibition of obesity development with combined use of a green tea powder, an investigation into the molecular pathways underlying catechin effect on energy and protein metabolism is crucial for more accurate dosage and dietary implementation.

In conclusion, the results of the present study indicate that feeding of 2% green tea in a high fat diet, reduces body weight by decreasing adipose tissue (e.g., stimulation of lipolysis; plasma NEFA elevation), promoting skeletal muscle mass, and increased hepatic β-oxidation (e.g., increased basal metabolic rate), via the synergistic effect of catechin and caffeine. Nevertheless, there is growing evidence to suggest that compounds found in food may also alter feeding behaviour (Scott, et al., 2008) and thus obesity development/ and successful weight loss. A summative discussion of this thesis in the next chapter will try to highlight the importance of viewing diseases such as obesity and cachexia in context of an inter-relationship rather than as separate conditions.

All limitations mentioned were due to lack of funding and time. The limitations of this study include the inability to conduct DEXA over three time points in the 18 weeks. A baseline and mid-way point would have been useful to determine each mouse's change in body weight and also at which point in the study any obesity protection from consuming green tea in particular occurred. Also it would have been useful to measure the total anti-oxidant capacity of the plasma and also the anti-oxidant

capacity of each feed. In doing so, direct conclusions relating to the presence of antioxidants and weight loss could have been made. It is possible that even though the dosage of raw material is 2% of the total feed, the amount of polyphenol in that 2% may have (1) varied between the different diets, and (2) the presence of different polyphenols may have either (3) no effect or a (4) direct weight loss effect. These analyses mentioned as limitations would be necessary to complete provide extra information for a publication. Also, gene analysis to describe what caused the loss of weight in the green tea group even though their feed intake remained constant would be necessary. I would propose analysis of skeletal muscle and liver analysis of UCP2 and UCP3. Also I would analyze fatty acid synthase and oxidation pathways by analyzing fatty acid synthase (mRNA), leptin and adiponectin levels in plasma.

Chapter 6 General Discussion

6.1 Overview

The general aim of this thesis was to characterise two novel models of congestive heart failure cachexia. In both instances, previous models were established for CHF but not CHF cachexia. In addition, to compare the effects of body wasting in these two models, to a model of diet induced obesity which was studied last. The development of DIO was examined to study the effects of consuming different anti-oxidant containing beverages (e.g., green tea) in feed and observe changes in body weight and composition.

The overall focus of this work was on the role of UCP 3 in skeletal muscle energy metabolism in cardiac Cachexia, and UCP3 was responsible for increased energy expenditure during CHF cachexia, and the role of green tea to block development of DIO. Specifically, it was hypothesised that reduction in feed intake due to CHF would cause weight loss (e.g., skeletal muscle) and this hypo-caloric feed intake would lead to the increase in UCP's, and in combination, decrease body weight independent of pair-feeding. It was further hypothesised that the inclusion of 2% green tea in a high fat diet would prevent development of DIO in mice.

A pilot sheep study of CHF was firstly developed, to characterize the rate of pacing and degree of muscle wasting that would occur, and secondly to observe any changes in feed intake that may underlie this weight loss. The diet and pacing regime were successful in the first study to induce wasting of lean leg tissue in the sheep. The next animal study investigated the role of SAR-Ang II in inducing weight loss and increased energy expenditure.

Lastly, the third study investigated the effects of feeding different beverages which were assumed to contain polyphenols on body composition changes in a mouse model of DIO (e.g., chow, high-fat diet).

6.2. Body composition response to RVP

Right ventricular pacing is long established as a method to induce congestive heart failure (Montgomery, et al., 1992). In the present study there was decreased weight to some degree through decreased leg lean mass. However, some propose that the CHF disease progression does not affect body composition (Ingle, et al., 2007), however, feed intake may be reduced (Vaisman, et al., 2004) and have a significant effect on the energy homeostasis in the induction of congestive heart failure in the sheep. There was no change in feed intake in the first study condudcted in this thesis, but there was increased energy expenditure and leg lean tissue loss. Similarly, the effect of pacing on energy expenditure is a matter of dispute (Farrell, et al., 2001; Poehlman, et al., 1994) as different investigators observe it to be either lower or higher in CHF patients displaying Cachexia.

In the first experimental study of this thesis, the body composition altering effects of RVP pacing in the sheep on a normal lucerne and oat chaff diet were determined and the effects on energy expenditure and inter-organ movement of amino acids investigated. Firstly, there was lean leg tissue wasting. This lead the project to examine probable cause and the results of this work demonstrated that pacing for 180bpm for 8 weeks causes increased energy expenditure. Furthermore, this increase in elevated energy expenditure was found to be independent of total calorie intake (e.g., sheep were pair fed).

Although some studies have shown that RVP causes elevation of energy expenditure and palmitate turnover (Lommi, et al., 1998), the present study found no evidence to suggest that the pacing regime had any effect on palmitate turnover or energy expenditure regulation. On the contrary, the RVP sheep had a negative body weight growth, even while experiencing slightly higher energy expenditure especially at week 8. This finding supports the hypothesis that RVP in sheep leads to weight loss and increased elevated energy expenditure, and rejects findings by (De Sousa, et al., 2002), who observed unchanged skeletal muscle metabolism in heart failure with voluntary activity. A possible cause of increased energy expenditure with only a slight change in body weight may have been higher amino acid (i.e., leucine) turnover as per observations by (Toth & Matthews, 2006). On the basis that the present RVP model causes lean tissue loss in the leg with slight weight loss, and increased energy expenditure, the model can be accepted for use in the study of muscle atrophy in RVP**.**

In the second component of the first experimental study, there were changes in organ weights and body adipose tissue deposits in the RVP sheep. Numerous reviewers have outlined the complex alterations in organ weights and adipose tissue that occur during CHF Cachexia (Krack, et al., 2005; Schulze, et al., 2005). (Wittels & Spann, 1968), observed gross hypertrophy of the left ventricle and reduced oxidation of long-chain fatty acids in CHF. This finding agrees with the present studies findings which demonstrate that during CHF there are alterations in adipose tissue metabolism (e.g., adipocyte proliferation/ suppression of fatty acid oxidation) and the animal actually become more ‘obese’. To benefit future studies, this study successfully identified important issues surrounding the experimental design, namely, use of a higher number of animals (for less variability), chemical composition of carcass to validate DEXA measurements, and analysis of other tissues for genes

involved in the regulation of energy metabolism. In this regard, the first experimental study of this thesis has been shown to be a valuable animal model for the study of CHF Cachexia. In summary, the use of mechanical pacing was discontinued for the next study, and a smaller animal model using hormone induced heart failure was used so more invasive measurements could be made.

6.3 Response of rat body weight and composition to infusion of SAR-Ang II

A number of animal studies has shown that infusion of Ang II induces heart failure and weight loss. The infusion of SAR-Ang II into Sprauge Dawley rats induced CHF and weight loss which is partially due to reduced feed intake and a smaller component that is independent from feed intake, arising from a metabolic factor (i.e., elevation in muscle and hepatic proton leak), like those observed by Cassis et al. (1998) (i.e., thermogenesis). Porter et al. (2003) notes that the elevation of thermogenesis is due to increased UCP1 in BAT. The renin-Angiotensin system is responsible for maintaining renal and organ perfusion and blood pressure (von Haehling, et al., 2007). However, very little is known about the origin of elevated energy expenditure and body compositional changes during the CHF wasting. The study described in Chapter 4 of this thesis is the first to directly investigate the effects of infusion of SAR-Ang II on body weight, body composition and, importantly, whether UCP3 in the skeletal muscle is largely responsible for elevated energy expenditure in the adult Sprauge Dawley rat. The findings presented in this work demonstrate that infusion of SAR-Ang II for a 5 day period is associated with more rapid body weight loss than observed in the pair fed group. This indicates that weight loss in the SAR-Ang II infused rats cannot be solely explained by reduction in feed intake. After examining weight loss by body composition, there was no specific

catabolism of the three components of body composition (i.e., muscle, fat, bone) to waste away. The weight loss was uniform across all three components. In this study, different methods of calculating body composition were used, which also aided in the estimation of energy expenditure. The SAR-Ang II rats had increased energy expenditure when corrected for body weight and more significantly when corrected for body protein. This finding indicated that the SAR-Ang II rats have a component of metabolism which is uncoupled from oxidative phosphorylation (e.g., increased proton leak from the inner mitochondrial membrane). After measuring UCP3 in the skeletal muscle in both studies there was no significant difference between treatment group and pair-fed group, even though energy expenditure was increased. It is concluded that UCP3 in skeletal muscle are not responsible for the uncoupling of phosphorylation in the two models mentioned above. Further, the infusion of SAR-Ang II directly induces heart failure and cardiac hypertrophy when compared with pair-fed control rats. This hypertrophy and thus increased cardiac output would have contributed to increased energy expenditure when compared to the pair-fed control group, thus explaining part of the body weight loss caused by increased energy expenditure.

This is the first evidence which shows that UCP 3 is not responsible for elevated energy expenditure in SAR-Ang II infused rats. Notably, the absence of increased UCP3 in the presence of SAR-Ang II induced CHF does not suggest that UCP's are not involved in elevation of energy expenditure, but calls for a more serious study of all genes that regulate energy metabolism in key metabolic tissues (e.g., liver, skeletal muscle, adipose tissue and heart). In addition, other tissues such as the liver should be studied as it is the primary site for a bulk of physiological energy metabolism. This is in contrast to other models of Ang II induced heart failure which

cite UCP1 and thermogenesis to be responsible for some of the increased energy expenditure (Porter & Potratz, 2004). In the present study, accelerated weight loss and elevated energy expenditure occurred only when SAR-Ang II was infused, not in pair feeding. In this context, the combined influence of the Ang II pathway to cause fullness and increase metabolism can be identified as the primary cause of wasting. However, the wasting was not discriminate to a certain part of the body composition which contradicts previous findings showing that Ang II specifically causes muscle specific.

The present study also investigated the recovery of rats after the cessation of SAR-Ang II infusion. It was hypothesised that after cessation of infusion, the rats would not regain weight to the same degree as the pair-fed individuals. Consistent with the present body of literature, re-feeding during and after the cachectic episode is not able to assist an individual to entirely re-gain as weight lost that occured during cachexia or particularily aging sarcopenia (Roberts, et al., 1994). Furthermore, in direct contrast, individuals with anorexia nervosa forced fed were able to regain weight previously lost (Thiels, 2008). On the other hand, the present study has found that increased appetite after cessation of SAR-Ang II infusion was not sufficient to produce full re-gain growth. Overall, these findings suggest a possible mechanisms for satiety in cardiac cachexia by exposure to SAR-Ang II infusion.

The presence of cardiac hypertrophy suggests that there was CHF present. However, further studies are required to properly test this hypothesis. The measurement of blood pressure or analysis of cytokine hormones (e.g., BNP or TNF-α), which correlated with increased blood pressure in CHF would be necessary to confirm this theory. (Pan, et al., 2004) have reviewed aspects of this hypothesis using CHF patients and found that TNF-α was quite useful as a marker to predict the

prognosis of CHF. Likewise, future studies using the present animal model could utilise feed supplements/ dietary modulation or exercise treatment to test whether supplementation of BCAA nutrition with omega-3 fatty acids and resistance training, may assist the rats to gain weight in the recovery period. Since green tea catechins have been used as dietary supplements in the use of promoting muscle deposition, they too could be used to blunt the sharp decline in body weight in the SAR-Ang II group during infusion. Also there is need for further study to identify key diagnostic markers of cardiac cachexia in this model. Decreased feed intake and body weight, and increased water intake, together do not fully describe fully cachexia. Screening of blood metabolites or gene analysis (i.e., fat and skeletal muscle metabolism markers) may be necessary to fully characterize the prognosis of the condition. One example may be using a radio-labelled leucine tracer as described by (Toth & Matthews, 2006) and examine protein turnover. In relation to direct rises in energy expenditure, it is possible to using indirect calorimetry as per (Cassis, et al., 2002), or abdominal temperature or hepatic UCP mRNA expression.

To fully characterize the role of Ang II in cardiac cachexia, further work is necessary to examine the activity of Ang II receptor signaling pathways and their influence to regulate energy expenditure. A wide variety of studies has demonstrated that Ang II works on a number of molecules to affect feed and water intake (Volmert, et al., 1991) and energy expenditure (Porter, et al., 2004). An investigation into the tissue specific expression of UCP's or other regulators of energy expenditure (e.g. other UCP's) and the activity of these key molecules is warranted to provide a more complete characterization of Ang II actions in both central and peripheral tissues to affect weight regulation.

6.4. The influence of green tea catechins on obesity regulation

In an extension of the two preceding animal studies investigating cardiac cachexia, an animal model of DIO was used to compare a different syndrome of body weight dysfunction and also for this DIO model to act as a precursor for heart failure (i.e., morbid obesity). This study was very unique in that anti-oxidant containing substances were delivered in the feed and not traditionally in the beverage format to correct dysfunction in body weight homeostasis caused by excessive calorie intake (e.g., fat and carbohydrates). Custom diets were made containing a realistic amount of antioxidant containing beverage (i.e., 2% or 20g in 1kg). The difference in body weight and composition was closely studied with measures of NEFA and plasma glucose. Overall, the results of the DIO study found that adding green tea to a high fat feed directly prevented mice from developing obesity.

Moreover, whilst conducting the diet induced model of obesity it was shown that the addition (i.e., 2%) of green tea in feed of mice reduced weight gain. No single polyphenol was identified to be attributable for the weight gain protection mechanism; however other authors suggest ECGC as been the most potent adipolytic catechin present in green tea (Diepvens, et al., 2007). Among the tissue-specific effects that were observed, feeding of green tea with a high fat diet appeared to have had the greatest effects on reducing adipose tissue and promoting skeletal muscle. Therefore, feeding 2% green-tea with a high fat diet actually protects against adipocyte deposition (e.g., via increased lipolysis, reduced fat absorption and suppression of lipogenesis) and promotes skeletal muscle growth. A possible mechanism to describe this hypothesis is that green tea has been found to limit weight regain after weight loss as green tea offsets the expected reduction in energy expenditure with decreased weight as energy expenditure is a function of lean mass (Diepvens, et al., 2007).

It is important to recognize that only a limited number of possible diets could be selected as the number of animals used for the study exceeded n = 60 animals. A chow based dietary treatment group may also have proved valuable to study the comparisons between the effects of green tea on a normal dietary versus obese regime as well. In this context, the present results indicate that eating any polyphenol containing beverage (other that green tea) in conjunction with a high fat diet had a relatively small impact to impede weight gain in DIO. Rather than causing modest or slight changes in body weight or composition, feeding of other beverages (i.e., cocoa) actually rivaled that of the control diet and was associated with extreme adiposity. Future, studies may benefit from this model as Japanese green tea powder has been shown to promote lean mass deposition and opens the door way to a whole series of experiments which identify the pathways catechins act on to drastically change body composition and energy expenditure.

Obesity is also linked to behavioural traits such as anxiety or depression. There is a correlation between the amount of adipose tissue and anxiety. Behaviour may impair an individual's ability to make informed dietary selection and quantities eaten, due to compulsive eating behaviour disorders and may contribute to co-morbidity of chronic depression and heart failure, thus possible cardiac cachexia (Bousoño M, 2008). This is an area of research which requires further examination and explanation of the inter-relationships with physiological outcomes of obesity and primary causes (e.g., dietary education, correcting habits and attitudes to food and lifestyle).

It is important to note, that both coffee and green tea contained caffeine which is a known stimulator of adipocyte metabolism (e.g., mobilization, turnover and oxidation) (Acheson, et al., 2004) and also elevates energy expenditure (Bracco, et al.,

1995). The variation within the chosen animals comprising the group to loss or re-gain weight and body weight composition may represent an idea for further investigation. The proposed study could tailor specific polyphenols, and a corresponding dose rate for a particular commencing phenotype (e.g., maybe assess the individuals body weight and body composition prior top recommending a certain polyphenol compound to prevent further diet induced obesity). Biomarkers may be used as tools to combat different weight altering diseases. A patient's individual susceptibility to develop obesity or cachexia may be diagnosed using screening of biomarkers. It would be advantageous to catagorize individual polyphenol's efficacy to induce a greater response to weight loss or body composition modulation per the individual's needs and patient health history, age, body composition. Simply prescribing a polyphenol containing food to all obese individuals is a haphazard approach to treatment. Dietary intake, rate of absorption of macro-nutrients and the proceeding metabolic processes until excretion all differ among individuals, thus gene expression of key pro-obesity genes may offer insight into a patient's ability to lose weight or inhibit weight gain given a certain polyphenol and dietary treatment.

In a review by (Spencer, et al., 2008), a number of factors surrounding this hypothesis are discussed. Analysis of the patient's diet for polyphenols, their re-occurrence in blood and urine would need a high level of specificity. Individuals often self-report diet and have different cooking methods, so the biomarker relationships would require clear framework for understanding these and other (e.g., impact of alteration at cooking, kinetics of absorption, polyphenol metabolism and excretion phase of the particular biomarker) factors (Spencer, et al., 2008). It may be possible to have a mathematical model where different numerals are assigned to different foods to describe their polyphenol function capacity to influence body weight. Based on

previous investigation, the inter-relationship between those foods containing polyphenols and those foods which may have opposing effects could be studied and a relationship could be derived. This relationship could predict the prognosis of weight loss and the precise quantification of polyphenol containing beverages to be used to gain the desired effect. The type and quantity of polyphenol may change as body weight is lost and body composition changes in line with blood metabolites.

If the level of green tea remains constant and the diet is low in fat, this dietary program may have only caused a modest decrease in body fat content and increase in plasma NEFA as can be observed if the mice were consuming a chow diet. However, if this dietary program is exposed to a high-fat diet, it would become more prominent, as this dietary program may greatly accelerate the lipolytic response of DIO, as can be seen in the green tea group of mice. This hypothesis is consistent with an emerging consensus on the direct action of green tea catechin with lipolytic enzymes and suppression of lipogenic enzymes, which suggests that rather than directly causing weight loss; green tea catechins facilitate anti-adipocyte influences (Murase, et al., 2002) and supports skeletal muscle growth after injury (Bordoni, et al., 2002).

Secondly, a decrease body weight in obesity (e.g., adipocyte number and size) suggest that there is a reduction in oxidative stress in peripheral tissues. It has been proposed, that increased oxidative stress during obesity is linked to a multitude of other effects, arising from impairment of mitochondrial function in skeletal muscle and is indirectly caused by insulin resistance and enhanced oxidative stress (Bonnard, et al., 2008). This particular hypothesis supports green tea catechin's ability to inhibit TNF-α expression and thus inflammation, oxidative damage and inhibit tumour-promoting activities of epidermal growth factor and NF-κβ (Huber, et al., 2003; Lamson, et al., 2001). It may be assumed that a decline in mitochondrial function may

induce slower metabolism, but the mitochondria may still uncouple respiration via UCP's and thus impede obesity development (e.g. green tea group). This hypothesis agrees with Dulloo, et al., (2000) who suggests that the main weight modulation effect of green tea is its thermogenic effect, generally attributed to its caffeine content (i.e., sympathetically released noradrenalin), stimulating hepatic β-oxidation activity (Kobayashi-Hattori, et al., 2005).

Overall, the observations made in the study have identified a number of key pathways that can be accepted and focused for future research; metabolic regulators of energy expenditure, lipolysis, lipogenesis, mitochondrial oxidative stress and biomarkers for efficacy of polyphenol use in treatment of obesity and cachexia. Caution must be warranted to investigate all levels of the study as the whole body changes must marry with metabolite levels and changes in gene expression. To test this hypothesis it will be necessary to measure oxidative capacity of the plasma, liver and muscle primary cell culture-lines, gene analysis of oxidative stress and protein function directly. Further, the levels of the native and introduced antioxidants should be analyzed and release of any factors that may be lipolytic should be observed to further understand the exact pathways (Garcia-Diaz, et al., 2008; Penfornis, et al., 2005).

6.5. Concluding Remarks and Future Directions

The principal goal of this thesis was to characterize models of cardiac cachexia to identify possible mechanisms by which the progression of the condition leads to satiety, weight loss (e.g., skeletal muscle) and compare them to DIO, to develop morbid obesity, a known precursor of CHF. The studies discussed in this thesis work have achieved the aims and were successfully developed models for further exploration of cardiac cachexia as a result of mechanical of hormonal induced

CHF. Furthermore, these studies identify elevated energy expenditure as a key mechanism through which weight loss was greater than feed intake and were not attributed at a molecular level to UCP3 in skeletal muscle. Further, the use of green tea in a high fat diet clearly displayed that the development of DIO could be prevented, and thus can also be used as a treatment for weight gain and promotion of skeletal muscle in cachexia. It is apparent that in both conditions there was body weight dysfunction and notable changes representing views from different ends of the spectrum of body weight, and composition.

These changes in body weight and composition highlight possible alterations in the regulation of anabolic and catabolic hormones and tissue response to different fuel substrates, their storage and utilization. Further, the work showed that catechins in conjunction with caffeine may be a means of reducing oxidative stress which may be a fundamental cause of obesity. Overall, the results presented in this thesis have identified a number of metabolite and molecular changes in animals with CHF induced cachexia. These findings demonstrate the need to study the inter-related mechanistic pathways which may be the underlying causes of body weight dysfunction, which lead further researchers to study the basis of mitochondrial control of both cachexia and obesity.

All limitations mentioned in this thesis were caused by lack of funding and time in particular. In discussing future directions that may be undertaken in each study, the main goal would be to complete the relevant analysis to a level in which data could be submitted as a publication(s). In Chapter 3, the study would have to be repeated with equal number of sheep to confirm beyond week 8, when wasting occurs by loss of lean leg tissue and body weight in particluar. The inter-organ amino acid analysis could be perfomed to examine whether there is specific loss of certain amino acids

(e.g., BCAA) such as hindlimb. Further analysis of UCP2 and UCP3 in the liver with other genes involved in energy regulation may prove useful to explain the exact pathways for elevated energy expenditure and weight loss in CHF cachexia. This also could be applied to the studies in Chapter 4 and Chapter 5. In Chapter 4, a group of n = 3 rats per group could be used to confirm body compositional changes using a small animal DEXA. Not only could the body composition be studied at different time points but also the exact moment of weight loss and gain and what tissues are involved would be invaluable information. Also the study in Chapter 5 could be included in the future study and the post-infusion AngII rats could receive a chow diet with green tea added. The green tea catechins may stimulate skeletal muscle growth and suppression of adipose tissue re-growth. In Chapter 5, the amount of polyphenol in the feed could be quantified and a dose trial conducted (i.e. 0, 1, 2 and 5% green tea in a high fat diet performed). This would confirm whether a higher level of green tea dosage would result in faster and more pronounced obesity protective effect in mice consuming a high fat diet with green tea. Overall there is a need to conduct some further work to confirm findings and publish work.

Chapter 7 References

Abid, Z. B., Feki, M., Hedhili, A., and Hamdaoui, M. H. (2007). Artemisia herba-alba Asso (Asteraceae) has equivalent effects to green and black tea decoctions on antioxidant processes and some metabolic parameters in rats. *Ann Nutr Metab, 51*(3), 216-222.

Acheson, K. J., Gremaud, G., Meirim, I., Montigon, F., Krebs, Y., Fay, L. B., Gay L.J, Schneiter P., Schindler C., Tappy L. (2004). Metabolic effects of caffeine in humans: lipid oxidation or futile cycling? *Am J Clin Nutr, 79*(1), 40-46.

Adolph, R. J., and Bliss, H. A. (1962). Body composition in experimental congestive heart failure. *Circ Res, 10*, 933-938.

Agustsson, T., Ryden, M., Hoffstedt, J., van Harmelen, V., Dicker, A., Laurencikiene, J., Isaksson B., Permert J., Arner P. (2007). Mechanism of increased lipolysis in cancer cachexia. *Cancer Res, 67*(11), 5531-5537.

Ailhaud, G., Fukamizu, A., Massiera, F., Negrel, R., Saint-Marc, P., and Teboul, M. (2000). Angiotensinogen, Angiotensin II and adipose tissue development. In*t J Obes Relat Metab Disord, 24 Suppl 4*, S33-35.

Akamizu, T., and Kangawa, K. (2007). Emerging results of anticatabolic therapy with ghrelin. *Curr Opin Clin Nutr Metab Care, 10*(3), 278-283.

Anderson, S. E., Cohen, P., Naumova, E. N., Jacques, P. F., and Must, A. (2007). Adolescent obesity and risk for subsequent major depressive disorder and anxiety disorder: prospective evidence. *Psychosom Med, 69*(8), 740-747.

Andreasson, A., Arborelius, L., Erlanson-Albertsson, C., and Lekander, M. (2007). A putative role for cytokines in the impaired appetite in depression. *Brain Behav Immun, 21*(2), 147-152.

Anker, S. D., Chua, T. P., Ponikowski, P., Harrington, D., Swan, J. W., Kox, W. J.,

Poole-Wilson P.A., Coats A.J. (1997). Hormonal changes and catabolic/anabolic imbalance in chronic heart failure and their importance for cardiac cachexia. *Circulation, 96*(2), 526-534.

Anker, S. D., & Coats, A. J. (1999). Cardiac cachexia: a syndrome with impaired survival and immune and neuroendocrine activation. *Chest, 115*(3), 836-847.

Anker, S. D., & Rauchhaus, M. (1999). Heart failure as a metabolic problem. *Eur J Heart Fail, 1*(2), 127-131.

Annida, B., and Stanely Mainzen Prince, P. (2004). Supplementation of fenugreek leaves lower lipid profile in streptozotocin-induced diabetic rats. *J Med Food, 7*(2), 153-156.

Aquilani, R., Opasich, C., Dossena, M., Iadarola, P., Gualco, A., Arcidiaco, P., Viglio, S., Boschi, F., Verri, M., Pasini, E. (2005). Increased skeletal muscle amino acid release with light exercise in deconditioned patients with heart failure. *J Am Coll Cardiol, 45*(1), 158-160.

Arciero, P. J., Gardner, A. W., Calles-Escandon, J., Benowitz, N. L., and Poehlman, E. T. (1995). Effects of caffeine ingestion on NE kinetics, fat oxidation, and energy expenditure in younger and older men. *Am J Physiol, 268*(6 Pt 1), E1192-1198.

Ardies, C. M. (2002). Exercise, cachexia, and cancer therapy: a molecular rationale. *Nutr Cancer, 42*(2), 143-157.

Argiles, J. M., Alvarez, B., and Lopez-Soriano, F. J. (1997). The metabolic basis of cancer cachexia. *Med Res Rev, 17*(5), 477-498.

Argiles, J. M., Lopez-Soriano, J., Almendro, V., Busquets, S., and Lopez-Soriano, F. J. (2005). Cross-talk between skeletal muscle and adipose tissue: a link with obesity? *Med Res Rev, 25*(1), 49-65.

Argiles, J. M., Lopez-Soriano, J., Busquets, S., and Lopez-Soriano, F. J. (1997).

Journey from cachexia to obesity by TNF. *Faseb J, 11*(10), 743-751.

Argiles, J. M., Moore-Carrasco, R., Busquets, S., and Lopez-Soriano, F. J. (2003). Catabolic mediators as targets for cancer cachexia. *Drug Discov Today, 8*(18), 838-844.

Asakawa, A., Inui, A., Kaga, T., Katsuura, G., Fujimiya, M., Fujino, M. A., Fujimiya, M., Fujino, M.A., Kasuga, M. (2003). Antagonism of ghrelin receptor reduces food intake and body weight gain in mice. *Gut, 52*(7), 947-952.

Ashida, H., Furuyashiki, T., Nagayasu, H., Bessho, H., Sakakibara, H., Hashimoto, T., Kanazawa, K. (2004). Anti-obesity actions of green tea: possible involvements in modulation of the glucose uptake system and suppression of the adipogenesis-related transcription factors. *Biofactors, 22*(1-4), 135-140.

Azhar, G., Wei, J.Y. (2006). Nutrition and cardiac cachexia. *Curr Opin Clin Nutr Metab Care. , 9*(1), 18-23.

Bacchi, F., Mathe, A. A., Jimenez, P., Stasi, L., Arban, R., Gerrard, P, Caberlotto, L. (2006). Anxiolytic-like effect of the selective neuropeptide Y Y2 receptor antagonist BIIE0246 in the elevated plus-maze. *Peptides, 27*(12), 3202-3207.

Bacon, L., Stern, J. S., Keim, N. L., and Van Loan, M. D. (2004). Low bone mass in premenopausal chronic dieting obese women. *Eur J Clin Nutr, 58*(6), 966-971.

Baracos, V. E., and Mackenzie, M. L. (2006). Investigations of branched-chain amino acids and their metabolites in animal models of cancer. *J Nutr, 136*(1 Suppl), 237S-242S.

Barber, M. D., Ross, J. A., Voss, A. C., Tisdale, M. J., and Fearon, K. C. (1999). The effect of an oral nutritional supplement enriched with fish oil on weight-loss in patients with pancreatic cancer. *Br J Cancer, 81*(1), 80-86.

Bartlett, D. L., Stein, T. P., & Torosian, M. H. (1995). Effect of growth hormone and protein intake on tumor growth and host cachexia. *Surgery, 117*(3), 260-267.

Beaufort-Krol, G. C., Takens, J., Smid, G. B., Molenkamp, M. C., Zijlstra, W. G., & Kuipers, J. R. (1999). Lower arterial glucose concentrations in lambs with aortopulmonary shunts after an 18-hour fast. *Metabolism, 48*(9), 1082-1088.

Beauvoit, B., Rigoulet, M., Bunoust, O., Raffard, G., Canioni, P., and Guerin, B. (1993). Interactions between glucose metabolism and oxidative phosphorylations on respiratory-competent Saccharomyces cerevisiae cells. *Eur J Biochem, 214*(1), 163-172.

Beck, S. A., and Tisdale, M. J. (2004). Effect of cancer cachexia on triacylglycerol/fatty acid substrate cycling in white adipose tissue. *Lipids, 39*(12), 1187-1189.

Beck, V., Jaburek, M., Demina, T., Rupprecht, A., Porter, R. K., Jezek, P., Pohl, E.E. (2007). Polyunsaturated fatty acids activate human uncoupling proteins 1 and 2 in planar lipid bilayers. *Faseb J, 21*(4), 1137-1144.

Belin, R. J., Sumandea, M. P., Kobayashi, T., Walker, L. A., Rundell, V. L., Urboniene, D., Yuzhakova, M., Ruch, S.H., Geenen, D.L., Solaro, R.J., de Tombe, P.P. (2006). Left ventricular myofilament dysfunction in rat experimental hypertrophy and congestive heart failure. *Am J Physiol Heart Circ Physiol, 291*(5), H2344-2353.

Belizario, J. E., Katz, M., Chenker, E., and Raw, I. (1991). Bioactivity of skeletal muscle proteolysis-inducing factors in the plasma proteins from cancer patients with weight loss. *Br J Cancer, 63*(5), 705-710.

Benton, D., and Donohoe, R. T. (1999). The effects of nutrients on mood. *Public Health Nutr, 2*(3A), 403-409.

Bernstein, R. D., Zhang, X., Zhao, G., Forfia, P., Tuzman, J., Ochoa, F., Vogel T,

Hintze T.H. (1997). Mechanisms of nitrate accumulation in plasma during pacing-induced heart failure in conscious dogs. *Nitric Oxide, 1*(5), 386-396.

Berry, C., & Clark, A. L. (2000). Catabolism in chronic heart failure. *Eur Heart J, 21*(7), 521-532.

Berryman, D. E., List, E. O., Kohn, D. T., Coschigano, K. T., Seeley, R. J., and Kopchick, J. J. (2006). Effect of growth hormone on susceptibility to diet-induced obesity. *Endocrinology, 147*(6), 2801-2808.

Bidel, S., Hu, G., Sundvall, J., Kaprio, J., and Tuomilehto, J. (2006). Effects of coffee consumption on glucose tolerance, serum glucose and insulin levels--a cross-sectional analysis. *Horm Metab Res, 38*(1), 38-43.

Bihoreau, C., Monnot, C., Davies, E., Teutsch, B., Bernstein, K.E., Corvol, P., Clauser, E. (1993). Mutation of Asp74 of the rat angiotensin II receptor confers changes in antagonist affinities and abolishes G-protein coupling. *Proc Natl Acad Sci U S A. , 90*(11), 5133-5137.

Bing, C., Brown, M., King, P., Collins, P., Tisdale, M.J., Williams, G. (2000). Increased gene expression of brown fat uncoupling protein (UCP)1 and skeletal muscle UCP2 and UCP3 in MAC16-induced cancer cachexia. *Cancer Res. , 60*(9), 2405-2410.

Bing, C., Russell, S., Becket, E., Pope, M., Tisdale, M. J., Trayhurn, P., Jenkins J.R. (2006). Adipose atrophy in cancer cachexia: morphologic and molecular analysis of adipose tissue in tumour-bearing mice. *Br J Cancer, 95*(8), 1028-1037.

Bing, C., Russell, S. T., Beckett, E. E., Collins, P., Taylor, S., Barraclough, R., Tisdale, M.J., Williams, G. (2002). Expression of uncoupling proteins-1, -2 and -3 mRNA is induced by an adenocarcinoma-derived lipid-mobilizing factor. *Br J Cancer, 86*(4), 612-618.

Bonnard, C., Durand, A., Peyrol, S., Chanseaume, E., Chauvin, M. A., Morio, B., Vidal, H., Rieusset, J. (2008). Mitochondrial dysfunction results from oxidative stress in the skeletal muscle of diet-induced insulin-resistant mice. *J Clin Invest, 118*(2), 789-800.

Bordoni, A., Hrelia, S., Angeloni, C., Giordano, E., Guarnieri, C., Caldarera, C. M., Biagi, P.L. (2002). Green tea protection of hypoxia/reoxygenation injury in cultured cardiac cells. *J Nutr Biochem, 13*(2), 103-111.

Bosaeus, I., Daneryd, P., and Lundholm, K. (2002). Dietary intake, resting energy expenditure, weight loss and survival in cancer patients. *J Nutr, 132*(11 Suppl), 3465S-3466S.

Bose, M., Lambert, J. D., Ju, J., Reuhl, K. R., Shapses, S. A., and Yang, C. S. (2008). The major green tea polyphenol, (-)-epigallocatechin-3-gallate, inhibits obesity, metabolic syndrome, and fatty liver disease in high-fat-fed mice. *J Nutr, 138*(9), 1677-1683.

Boss, O., Hagen, T., and Lowell, B. B. (2000). Uncoupling proteins 2 and 3: potential regulators of mitochondrial energy metabolism. *Diabetes, 49*(2), 143-156.

Bossola, M., Muscaritoli, M., Costelli, P., Bellantone, R., Pacelli, F., Busquets, S., Argilès, J., Lopez-Soriano, F.J., Civello, I.M., Baccino, F.M., Rossi Fanelli, F., Doglietto, G.B. (2001). Increased muscle ubiquitin mRNA levels in gastric cancer patients. *Am J Physiol Regul Integr Comp Physiol, 280*(5), R1518-1523.

Bouayed, J., Rammal, H., Dicko, A., Younos, C., and Soulimani, R. (2007). Chlorogenic acid, a polyphenol from Prunus domestica (Mirabelle), with coupled anxiolytic and antioxidant effects. *J Neurol Sci, 262*(1-2), 77-84.

Bourdel-Marchasson, I., and Emeriau, J. P. (2001). Nutritional strategy in the management of heart failure in adults. *Am J Cardiovasc Drugs, 1*(5), 363-373.

Bousoño M, B. E., Alvarez E, Eguiluz I, Martín M, Roca M, Urretavizcaya M. (2008). Consequences of the long-term depression *Actas Esp Psiquiatr, 36*(2), 44-52

Bozzetti, F., Gavazzi, C., Ferrari, P., and Dworzak, F. (2000). Effect of total parenteral nutrition on the protein kinetics of patients with cancer cachexia. *Tumori, 86*(5), 408-411.

Bozzetti, F., Gavazzi, C., Mariani, L., and Crippa, F. (1999). Artificial nutrition in cancer patients: which route, what composition? *World J Surg, 23*(6), 577-583.

Bracco, D., Ferrarra, J. M., Arnaud, M. J., Jequier, E., and Schutz, Y. (1995). Effects of caffeine on energy metabolism, heart rate, and methylxanthine metabolism in lean and obese women. *Am J Physiol, 269*(4 Pt 1), E671-678.

Brand, M. D., Brindle, K. M., Buckingham, J. A., Harper, J. A., Rolfe, D. F., and Stuart, J. A. (1999). The significance and mechanism of mitochondrial proton conductance. In*t J Obes Relat Metab Disord, 23 Suppl 6*, S4-11.

Brand, M. D., Chien, L. F., Ainscow, E. K., Rolfe, D. F., and Porter, R. K. (1994). The causes and functions of mitochondrial proton leak. *Biochim Biophys Acta, 1187*(2), 132-139.

Bremner, J. D., Vythilingam, M., Vermetten, E., Nazeer, A., Adil, J., Khan, S., Staib, L.H., Charney, D.S. (2002). Reduced volume of orbitofrontal cortex in major depression. *Biol Psychiatry, 51*(4), 273-279.

Brink, M., Anwar, A., and Delafontaine, P. (2002). Neurohormonal factors in the development of catabolic/anabolic imbalance and cachexia. In*t J Cardiol, 85*(1), 111-121, discussion 121-114.

Brink, M., Chrast, J., Price, S. R., Mitch, W. E., and Delafontaine, P. (1999). Angiotensin II stimulates gene expression of cardiac insulin-like growth factor i and its receptor through effects on blood pressure and food intake. *Hypertension, 34*(5),

1053-1059.

Brink, M., Price, S. R., Chrast, J., Bailey, J. L., Anwar, A., Mitch, W. E., Delafontaine, P. (2001). Angiotensin II induces skeletal muscle wasting through enhanced protein degradation and down-regulates autocrine insulin-like growth factor I. *Endocrinology, 142*(4), 1489-1496.

Brink, M., Wellen, J., and Delafontaine, P. (1996). Angiotensin II causes weight loss and decreases circulating insulin-like growth factor I in rats through a pressor-independent mechanism. *J Clin Invest, 97*(11), 2509-2516.

Broca, C., Breil, V., Cruciani-Guglielmacci, C., Manteghetti, M., Rouault, C., Derouet, M., Rizkalla, S., Pau, B., Petit, P., Ribes, G., Ktorza, A., Gross, R., Reach, G, Taouis, M. (2004). Insulinotropic agent ID-1101 (4-hydroxyisoleucine) activates insulin signaling in rat. *Am J Physiol Endocrinol Metab, 287*(3), E463-471.

Brodan, V., Fabián, J., Andel, M., Pechar, J. (1978). Myocardial amino acid metabolism in patients with chronic ischemic heart disease. *Basic Res Cardiol , 73*(2), 160-170.

Brookes, P. S., Buckingham, J. A., Tenreiro, A. M., Hulbert, A. J., and Brand, M. D. (1998). The proton permeability of the inner membrane of liver mitochondria from ectothermic and endothermic vertebrates and from obese rats: correlations with standard metabolic rate and phospholipid fatty acid composition. *Comp Biochem Physiol B Biochem Mol Biol, 119*(2), 325-334.

Brookes, P. S., Zhang, J., Dai, L., Zhou, F., Parks, D. A., Darley-Usmar, V. M., Anderson,P.G. (2001). Increased sensitivity of mitochondrial respiration to inhibition by nitric oxide in cardiac hypertrophy. *J Mol Cell Cardiol, 33*(1), 69-82.

Browning, L. M. (2003). n-3 Polyunsaturated fatty acids, inflammation and obesity-related disease. *Proc Nutr Soc, 62*(2), 447-453.

Bruinsma, K., and Taren, D. L. (1999). Chocolate: food or drug? *J Am Diet Assoc, 99*(10), 1249-1256.

Burnham, J. M., Shults, J., Semeao, E., Foster, B. J., Zemel, B. S., Stallings, V. A., Leonard, M.B. (2005). Body-composition alterations consistent with cachexia in children and young adults with Crohn disease. *Am J Clin Nutr, 82*(2), 413-420.

Busquets, S., Almendro, V., Barreiro, E., Figueras, M., Argilés, J.M., López-Soriano, F.J. (2005). Activation of UCPs gene expression in skeletal muscle can be independent on both circulating fatty acids and food intake. Involvement of ROS in a model of mouse cancer cachexia. *FEBS Lett. , 579*(3), 717-722.

Busquets, S., Alvarez, B., Llovera, M., Agell, N., Lopez-Soriano, F. J., and Argiles, J. M. (2000). Branched-chain amino acids inhibit proteolysis in rat skeletal muscle: mechanisms involved. *J Cell Physiol, 184*(3), 380-384.

Busquets, S., Figueras, M. T., Fuster, G., Almendro, V., Moore-Carrasco, R., Ametller, E., Argilés, J.M., López-Soriano, F.J. (2004). Anticachectic effects of formoterol: a drug for potential treatment of muscle wasting. *Cancer Res, 64*(18), 6725-6731.

Busquets, S., Garcia-Martinez, C., Alvarez, B., Carbo, N., Lopez-Soriano, F. J., and Argiles, J. M. (2000). Calpain-3 gene expression is decreased during experimental cancer cachexia. *Biochim Biophys Acta, 1475*(1), 5-9.

Cabal-Manzano, R., Bhargava, P., Torres-Duarte, A., Marshall, J., Bhargava, P., and Wainer, I. W. (2001). Proteolysis-inducing factor is expressed in tumours of patients with gastrointestinal cancers and correlates with weight loss. *Br J Cancer, 84*(12), 1599-1601.

Cabassi, A., Coghi, P., Govoni, P., Barouhiel, E., Speroni, E., Cavazzini, S., Cantoni, A.M., Scandroglio, R., Fiaccadori, E. (2005). Sympathetic modulation by carvedilol

and losartan reduces Angiotensin II-mediated lipolysis in subcutaneous and visceral fat. *J Clin Endocrinol Metab, 90*(5), 2888-2897.

Cadenas, E., and Davies, K. J. (2000). Mitochondrial free radical generation, oxidative stress, and aging. *Free Radic Biol Med, 29*(3-4), 222-230.

Caligiani, A., Cirlini, M., Palla, G., Ravaglia, R., and Arlorio, M. (2007). GC-MS detection of chiral markers in cocoa beans of different quality and geographic origin. *Chirality, 19*(4), 329-334.

Cariuk, P., Lorite, M. J., Todorov, P. T., Field, W. N., Wigmore, S. J., and Tisdale, M. J. (1997). Induction of cachexia in mice by a product isolated from the urine of cachectic cancer patients. *Br J Cancer, 76*(5), 606-613.

Cassis, L., Helton, M., English, V., and Burke, G. (2002). Angiotensin II regulates oxygen consumption. *Am J Physiol Regul Integr Comp Physiol, 282*(2), R445-453.

Cassis, L. A. (2000). Fat cell metabolism: insulin, fatty acids, and renin. *Curr Hypertens Rep, 2*(2), 132-138.

Cassis, L. A., Marshall, D. E., Fettinger, M. J., Rosenbluth, B., and Lodder, R. A. (1998). Mechanisms contributing to Angiotensin II regulation of body weight. *Am J Physiol, 274*(5 Pt 1), E867-876.

Cazeau, S., Ritter, P., Bakdach, S., Lazarus, A., Limousin, M., Henao, L., Henao, L., Mundler, O., Daubert, J.C., Mugica, J. (1994). Four chamber pacing in dilated cardiomyopathy. *Pacing Clin Electrophysiol, 17*(11 Pt 2), 1974-1979.

Cederholm, T., Wretlind, B., Hellstrom, K., Andersson, B., Engstrom, L., Brismar, K., Scheynius, A., Forslid, J., Palmblad, J. (1997). Enhanced generation of interleukins 1 beta and 6 may contribute to the cachexia of chronic disease. *Am J Clin Nutr, 65*(3), 876-882.

Chabi, B., Ljubicic, V., Menzies, K. J., Huang, J. H., Saleem, A., and Hood, D. A.

(2008). Mitochondrial function and apoptotic susceptibility in aging skeletal muscle. *Aging Cell, 7*(1), 2-12.

Chabrashvili, T., Kitiyakara, C., Blau, J., Karber, A., Aslam, S., Welch, W. J., Wilcox, C.S. (2003). Effects of Angiotensin II type 1 and 2 receptors on oxidative stress, renal NADPH oxidase, and SOD expression. *Am J Physiol Regul Integr Comp Physiol, 285*(1), R117-124.

Chaloupecký, V., Hucín, B., Tláskal, T., Kostelka, M., Kucera, V., Janousek, J., Skovránek, J., Sprongl, L. (1997). Nitrogen balance, 3-methylhistidine excretion, and plasma amino acid profile in infants after cardiac operations for congenital heart defects: the effect of early nutritional support. *J Thorac Cardiovasc Surg. , 114*(6), 1053-1060.

Chan, F. K., Sung, J. J., Ma, K. M., Leung, Y. L., and Yeung, V. T. (1999). Protein-losing enteropathy in congestive heart failure: diagnosis by means of a simple method. *Hepatogastroenterology, 46*(27), 1816-1818.

Chen, L., Lee, M. J., Li, H., and Yang, C. S. (1997). Absorption, distribution, elimination of tea polyphenols in rats. *Drug Metab Dispos, 25*(9), 1045-1050.

Cheung, W. T., Lee, C. M., and Ng, T. B. (1988). Potentiation of the anti-lipolytic effect of 2-chloroadenosine after chronic caffeine treatment. *Pharmacology, 36*(5), 331-339.

Choo, J. J. (2003). Green tea reduces body fat accretion caused by high-fat diet in rats through beta-adrenoceptor activation of thermogenesis in brown adipose tissue. *J Nutr Biochem, 14*(11), 671-676.

Chow, E., Woodard, J. C., and Farrar, D. J. (1990). Rapid ventricular pacing in pigs: an experimental model of congestive heart failure. *Am J Physiol, 258*(5 Pt 2), H1603-1605.

Chu, K. Y., and Leung, P. S. (2007). Angiotensin II Type 1 receptor antagonism mediates uncoupling protein 2-driven oxidative stress and ameliorates pancreatic islet beta-cell function in young Type 2 diabetic mice. *Antioxid Redox Signal, 9*(7), 869-878.

Cicoira, M., Zanolla, L., Franceschini, L., Rossi, A., Golia, G., Zamboni, M., Tosoni, P., Zardini, P. (2001). Skeletal muscle mass independently predicts peak oxygen consumption and ventilatory response during exercise in noncachectic patients with chronic heart failure. *J Am Coll Cardiol, 37*(8), 2080-2085.

Cissik, J. H., Ehler, W. J., Hankins, G. D., & Snyder, R. R. (1991). Cardiopulmonary reference standards in the pregnant sheep (Ovis aries): a comparative study of ovine and human physiology in obstetrics. *Comp Biochem Physiol A Comp Physiol, 100*(4), 877-880.

Clapham, J. C., Arch, J. R., Chapman, H., Haynes, A., Lister, C., Moore, G. B., Piercy, V., Carter, S.A., Lehner, I., Smith, S.A., Beeley, L.J., Godden, R.J., Herrity, N., Skehel, M., Changani, K.K., Hockings, P.D., Reid, D.G., Squires, S.M., Hatcher, J., Trail, B., Latcham, J., Rastan, S., Harper, A.J., Cadenas, S., Buckingham, J.A., Brand, M.D., Abuin, A. (2000). Mice overexpressing human uncoupling protein-3 in skeletal muscle are hyperphagic and lean. *Nature, 406*(6794), 415-418.

Cocchi, M., Siniscalchi, C., Rogato, F., and Valeriani, A. (1983). Free fatty acid levels in habitual coffee drinkers in relation to quantities consumed, sex and age. *Ann Nutr Metab, 27*(6), 477-480.

Coletti, D., and Adamo, S. (2008). 'Highlights on Cachexia, from the 4th Cachexia Conference Tampa (FL), 6-9 Dec 2007'. *Basic Applied Myology, 18(5),* 109-114.

Colin-Ramirez, E., Castillo-Martinez, L., Orea-Tejeda, A., Asensio Lafuente, E., Torres Villanueva, F., Rebollar Gonzalez, V., Narváez David R, Dorantes García J.

(2006). Body composition and echocardiographic abnormalities associated to anemia and volume overload in heart failure patients. *Clin Nutr, 25*(5), 746-757.

Collins, P., Bing, C., McCulloch, P., and Williams, G. (2002). Muscle UCP-3 mRNA levels are elevated in weight loss associated with gastrointestinal adenocarcinoma in humans. *Br J Cancer, 86*(3), 372-375.

Conlisk, A. J., and Galuska, D. A. (2000). is caffeine associated with bone mineral density in young adult women? *Prev Med, 31*(5), 562-568.

Cordopatis, P., Manessi-Zoupa, E., Theodoropoulos, D., Bossé, R., Bouley, R., Gagnon, S., Escher, E. (1994). Methylation in positions 1 and 7 of angiotensin II. A structure-activity relationship study. *Int J Pept Protein Res.* , 44(4), 320-324.

Correia, M. L., and Haynes, W. G. (2004). Leptin, obesity and cardiovascular disease. *Curr Opin Nephrol Hypertens, 13*(2), 215-223.

Costa, G. (1977). Cachexia, the metabolic component of neoplastic diseases. *Cancer Res, 37*(7 Pt 2), 2327-2335.

Cottam, D. R., Mattar, S. G., Barinas-Mitchell, E., Eid, G., Kuller, L., Kelley, D. E., Schauer, P.R. (2004). The chronic inflammatory hypothesis for the morbidity associated with morbid obesity: implications and effects of weight loss. *Obes Surg, 14*(5), 589-600.

Crespy, V., and Williamson, G. (2004). A review of the health effects of green tea catechins in in vivo animal models. *J Nutr, 134*(12 Suppl), 3431S-3440S.

Curhan, G. C., Willett, W.C., Rimm, E.B., Spiegelman, D., Stampfer, M.J. (1996). Prospective study of beverage use and the risk of kidney stones. *Am J Epidemiol.* , *143*(3), 240-247.

Curtis, J. P., Selter, J. G., Wang, Y., Rathore, S. S., Jovin, I. S., Jadbabaie, F., Kosiborod, M., Portnay, E.L., Sokol, S.I., Bader, F., Krumholz, H.M. (2005). The

obesity paradox: body mass index and outcomes in patients with heart failure. *Arch Intern Med, 165*(1), 55-61.

Dakam, W., Shang, J., Agbor, G., and Oben, J. (2007). Effects of sodium bicarbonate and albumin on the in vitro water-holding capacity and some physiological properties of Trigonella foenum graecum L. galactomannan in rats. *J Med Food, 10*(1), 169-174.

Dallard, I., Cathebras, P., Sauron, C., and Massoubre, C. (2001) [Is cocoa a psychotropic drug? Psychopathologic study of a population of subjects self-identified as chocolate addicts]. *Encephale, 27*(2), 181-186.

Danesh, J., Muir, J., Wong, Y. K., Ward, M., Gallimore, J. R., and Pepys, M. B. (1999). Risk factors for coronary heart disease and acute-phase proteins. A population-based study. *Eur Heart J, 20*(13), 954-959.

Davos, C. H., Doehner, W., Rauchhaus, M., Cicoira, M., Francis, D. P., Coats, A. J., Clark, A.L,, Anker, S.D. (2003). Body mass and survival in patients with chronic heart failure without cachexia: the importance of obesity. *J Card Fail, 9*(1), 29-35.

de Lorgeril, M., and Salen, P. (2006). Selenium and antioxidant defenses as major mediators in the development of chronic heart failure. *Heart Fail Rev, 11*(1), 13-17.

De Sousa, E., Lechene, P., Fortin, D., N'Guessan, B., Belmadani, S., Bigard, X., Veksler, V., Ventura-Clapier, R. (2002). Cardiac and skeletal muscle energy metabolism in heart failure: beneficial effects of voluntary activity. *Cardiovasc Res, 56*(2), 260-268.

Dehghan, M., Akhtar-Danesh, N., and Merchant, A. T. (2005). Childhood obesity, prevalence and prevention. *Nutr J, 4*, 24.

Delafontaine, P., and Akao, M. (2006). Angiotensin II as candidate of cardiac cachexia. *Curr Opin Clin Nutr Metab Care, 9*(3), 220-224.

Delafontaine, P., and Brink, M. (2000). The growth hormone and insulin-like growth

factor 1 axis in heart failure. *Ann Endocrinol (Paris), 61*(1), 22-26.

Denaro, C. P., Brown, C. R., Jacob, P., 3rd, and Benowitz, N. L. (1991). Effects of caffeine with repeated dosing. *Eur J Clin Pharmacol, 40*(3), 273-278.

Devasena, T., and Venugopal Menon, P. (2007). Fenugreek seeds modulate 1,2-dimethylhydrazine-induced hepatic oxidative stress during colon carcinogenesis. It*al J Biochem, 56*(1), 28-34.

Devi, B. A., Kamalakkannan, N., and Prince, P. S. (2003). Supplementation of fenugreek leaves to diabetic rats. Effect on carbohydrate metabolic enzymes in diabetic liver and kidney. *Phytother Res, 17*(10), 1231-1233.

Devine, A., Hodgson, J. M., Dick, I. M., and Prince, R. L. (2007). Tea drinking is associated with benefits on bone density in older women. *Am J Clin Nutr, 86*(4), 1243-1247.

Diepvens, K., Westerterp, K.R., Westerterp-Plantenga, M.S. (2007). Obesity and thermogenesis related to the consumption of caffeine, ephedrine, capsaicin, and green tea. *Am J Physiol Regul Integr Comp Physiol. , 292*(1), R77-85.

Dobrian, A. D., Davies, M. J., Schriver, S. D., Lauterio, T. J., and Prewitt, R. L. (2001). Oxidative stress in a rat model of obesity-induced hypertension. *Hypertension, 37*(2 Part 2), 554-560.

Dong, F., Li, Q., Sreejayan, N., Nunn, J. M., and Ren, J. (2007). Metallothionein prevents high-fat diet induced cardiac contractile dysfunction: role of peroxisome proliferator activated receptor gamma coactivator 1alpha and mitochondrial biogenesis. *Diabetes, 56*(9), 2201-2212.

Dorri, Y., Sabeghi, M., and Kurien, B. T. (2007). Awaken olfactory receptors of humans and experimental animals by coffee odourants to induce appetite. *Med Hypotheses, 69*(3), 508-509.

Drevets, W. C. (2007). Orbitofrontal cortex function and structure in depression. *Ann N Y Acad Sci, 1121*, 499-527.

Droge, W., Hack, V., Breitkreutz, R., Holm, E., Shubinsky, G., Schmid, E., Galter, D. (1998). Role of cysteine and glutathione in signal transduction, immunopathology and cachexia. *Biofactors, 8*(1-2), 97-102.

Dulloo, A. G., Duret, C., Rohrer, D., Girardier, L., Mensi, N., Fathi, M., Chantre, P., Vandermander, J. (1999). Efficacy of a green tea extract rich in catechin polyphenols and caffeine in increasing 24-h energy expenditure and fat oxidation in humans. *Am J Clin Nutr, 70*(6), 1040-1045.

Dulloo, A. G., and Samec, S. (2001). Uncoupling proteins: their roles in adaptive thermogenesis and substrate metabolism reconsidered. *Br J Nutr, 86*(2), 123-139.

Dulloo, A. G., Seydoux, J., Girardier, L., Chantre, P., and Vandermander, J. (2000). Green tea and thermogenesis: interactions between catechin-polyphenols, caffeine and sympathetic activity. *Int J Obes Relat Metab Disord, 24*(2), 252-258.

Dzemali, O., Bakhtiary, F., Wittlinger, T., Dogan, S., Ackermann, H., Pitschner, H. F., Moritz, A., Kleine, P. (2007). Hemodynamic effects of left ventricular pacing site in an animal model of heart failure. *Thorac Cardiovasc Surg, 55*(8), 481-484.

Dziedzic, B., Szemraj, J., Bartkowiak, J., and Walczewska, A. (2007). Various dietary fats differentially change the gene expression of neuropeptides involved in body weight regulation in rats. *J Neuroendocrinol, 19*(5), 364-373.

Echtay, K. S., Winkler, E., Frischmuth, K., and Klingenberg, M. (2001). Uncoupling proteins 2 and 3 are highly active H(+) transporters and highly nucleotide sensitive when activated by coenzyme Q (ubiquinone). *Proc Natl Acad Sci U S A, 98*(4), 1416-1421.

Edelman, M. J., Gandara, D. R., Meyers, F. J., Ishii, R., O'Mahony, M., Uhrich, M.,

Lauder, I., Houston, J., Gietzen, D.W. (1999). Serotonergic blockade in the treatment of the cancer anorexia-cachexia syndrome. *Cancer, 86*(4), 684-688.

Eichhorn, E. J., and Bristow, M. R. (1996). Medical therapy can improve the biological properties of the chronically failing heart. A new era in the treatment of heart failure. *Circulation, 94*(9), 2285-2296.

Emery, P. W. (1999). Cachexia in experimental models. *Nutrition, 15*(7-8), 600-603.

Emre, Y., Hurtaud, C., Karaca, M., Nubel, T., Zavala, F., and Ricquier, D. (2007). Role of uncoupling protein UCP2 in cell-mediated immunity: how macrophage-mediated insulitis is accelerated in a model of autoimmune diabetes. *Proc Natl Acad Sci U S A, 104*(48), 19085-19090.

Engelen, M. P., Wouters, E. F., Deutz, N. E., Does, J. D., and Schols, A. M. (2001). Effects of exercise on amino acid metabolism in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med, 163*(4), 859-864.

Engeli, S., Schling, P., Gorzelniak, K., Boschmann, M., Janke, J., Ailhaud, G., Teboul, M., Massiéra, F., Sharma, A.M. (2003). The adipose-tissue renin-Angiotensin-aldosterone system: role in the metabolic syndrome? *Int J Biochem Cell Biol, 35*(6), 807-825.

Engeli, S., and Sharma, A. M. (2000). Role of adipose tissue for cardiovascular-renal regulation in health and disease. *Horm Metab Res, 32*(11-12), 485-499.

English, V., Cassis, L. (1999). Facilitation of sympathetic neurotransmission contributes to angiotensin regulation of body weight. *J Neural Transm , 106*(7-8), 631-644.

Ewart, H. S., Jois, M., and Brosnan, J. T. (1992). Rapid stimulation of the hepatic glycine-cleavage system in rats fed on a single high-protein meal. *Biochem J, 283 (Pt 2)*, 441-447.

Evans, R. K., Schwartz, D. D., & Gladden, L. B. (2003). Effect of myocardial volume overload and heart failure on lactate transport into isolated cardiac myocytes. *J Appl Physiol, 94*(3), 1169-1176.

Falconer, J. S., Fearon, K. C., Plester, C. E., Ross, J. A., and Carter, D. C. (1994). Cytokines, the acute-phase response, and resting energy expenditure in cachectic patients with pancreatic cancer. *Ann Surg, 219*(4), 325-331.

Farrell, A. G., Schamberger, M. S., Olson, I. L., and Leitch, C. A. (2001). Large left-to-right shunts and congestive heart failure increase total energy expenditure in infants with ventricular septal defect. *Am J Cardiol, 87*(9), 1128-1131, A1110.

Fedak, P. W., Verma, S., Weisel, R. D., and Li, R. K. (2005). Cardiac remodeling and failure: from molecules to man (Part I). *Cardiovasc Pathol, 14*(1), 1-11.

Figueroa, J. E., Vijayagopal, P., Prasad, A., Schapira, D. V., and Prasad, C. (1999). Isolation, characterization, and distribution of a 24-kDa proteoglycan in the urine of cachectic cancer and AIDS patients. *Biochem Biophys Res Commun, 254*(3), 642-646.

Fine, J. (1935). The biuret method of estimating albumin and globulin in serum and urine. *Biochem J, 29*(3), 799-803.

Fiorini, R. N., Donovan, J. L., Rodwell, D., Evans, Z., Cheng, G., May, H. D., Milliken, C.E., Markowitz, J.S., Campbell, C., Haines, J.K., Schmidt, M.G., Chavin, K.D. (2005). Short-term administration of (-)-epigallocatechin gallate reduces hepatic steatosis and protects against warm hepatic ischemia/reperfusion injury in steatotic mice. *Liver Transpl, 11*(3), 298-308.

Forman-Hoffman, V. L., Ruffin, T., Schultz, S.K. (2006). Basal metabolic rate in anorexia nervosa patients: using appropriate predictive equations during the refeeding process. *Ann Clin Psychiatry.* , 18(2), 123-127.

Frayn, K.N. (1991). 'Insulin resistance in obesity and wasting disorders', in Stock,

M.J., and Rothwell, N.J. (1991). ‘Obesity and Cachexia: Physiological Mechanisms and New Approaches to Pharmacological Control’. John Wiley and Sons Publishing, Chichester. U.K.

Friedman, M. I. (1998). Fuel partitioning and food intake. *Am J Clin Nutr, 67*(3 Suppl), 513S-518S.

Fukunaga, Y., Itoh, H., Hosoda, K., Doi, K., Matsuda, J., Son, C., Yamashita, J., Chun, T.H., Tanaka, T., Inoue, M., Masatsugu, K., Saito, T., Sawada, N., Nakao, K. (2000). Altered gene expression of uncoupling protein-2 and -3 in stroke-prone spontaneously hypertensive rats. *J Hypertens, 18*(9), 1233-1238.

Fukushima, Y., and Kumagai, A. (2006). [Prevention of osteoporosis by foods and dietary supplements. Chocolate malt drink MILO: nutrition in children and calcium absorption]. *Clin Calcium, 16*(10), 1706-1713.

Fulle, S., Belia, S., and Di Tano, G. (2005). Sarcopenia is more than a muscular deficit. *Arch ital Biol, 143*(3-4), 229-234.

Fulle, S., Protasi, F., Di Tano, G., Pietrangelo, T., Beltramin, A., Boncompagni, S., Vecchiet, L., Fanò, G. (2004). The contribution of reactive oxygen species to sarcopenia and muscle ageing. *Exp Gerontol, 39*(1), 17-24.

Galinier, M., Pathak, A., Roncalli, J., and Massabuau, P. (2005). [Obesity and cardiac failure]. *Arch Mal Coeur Vaiss, 98*(1), 39-45.

Garcia, J. M., Garcia-Touza, M., Hijazi, R. A., Taffet, G., Epner, D., Mann, D., Smith, R.G., Cunningham, G.R., Marcelli, M. (2005). Active ghrelin levels and active to total ghrelin ratio in cancer-induced cachexia. *J Clin Endocrinol Metab, 90*(5), 2920-2926.

Garcia-Diaz, D. F., Campion, J., Milagro, F. I., Paternain, L., Solomon, A., and Martinez, J. A. (2008). Ascorbic acid oral treatment modifies lipolytic response and

behavioural activity but not glucocorticoid metabolism in cafeteria diet fed rats. *Acta Physiol (Oxf)*.

Garlid, K. D., Jaburek, M., Jezek, P., and Varecha, M. (2000). How do uncoupling proteins uncouple? *Biochim Biophys Acta, 1459*(2-3), 383-389.

George, I., Xydas, S., Mancini, D. M., Lamanca, J., DiTullio, M., Marboe, C. C., Shane, E., Schulman, A.R., Colley, P.M., Petrilli, C.M., Naka, Y., Oz, M.C., Maybaum, S. (2006). Effect of clenbuterol on cardiac and skeletal muscle function during left ventricular assist device support. *J Heart Lung Transplant, 25*(9), 1084-1090.

Gibbons, M. J., Gilchrist, N. L., Frampton, C., Maguire, P., Reilly, P. H., March, R. L., Wall, C.R. (2004). The effects of a high calcium dairy food on bone health in pre-pubertal children in New Zealand. *Asia Pac J Clin Nutr, 13*(4), 341-347.

Goldberg, A. L., Kettelhut, I. C., Furuno, K., Fagan, J. M., and Baracos, V. (1988). Activation of protein breakdown and prostaglandin E2 production in rat skeletal muscle in fever is signaled by a macrophage product distinct from interleukin 1 or other known monokines. *J Clin Invest, 81*(5), 1378-1383.

Gomes-Marcondes, M. C., Smith, H. J., Cooper, J. C., and Tisdale, M. J. (2002). Development of an in-vitro model system to investigate the mechanism of muscle protein catabolism induced by proteolysis-inducing factor. *Br J Cancer, 86*(10), 1628-1633.

Gompf, R. E. (2005). Nutritional and herbal therapies in the treatment of heart disease in cats and dogs. *J Am Anim Hosp Assoc, 41*(6), 355-367.

Gordon, J. N., Green, S. R., and Goggin, P. M. (2005). Cancer cachexia. *Qjm, 98*(11), 779-788.

Gornall, A. G., Bardawill, C. J., and David, M. M. (1949). Determination of serum

proteins by means of the biuret reaction. *J Biol Chem*, *177*(2), 751-766.

Grady, K. L., Naftel, D., Pamboukian, S. V., Frazier, O. H., Hauptman, P., Herre, J., Eisen, H., Smart, F., Bourge, R. (2005). Post-operative obesity and cachexia are risk factors for morbidity and mortality after heart transplant: multi-institutional study of post-operative weight change. *J Heart Lung Transplant*, *24*(9), 1424-1430.

Graham, H. N. (1992). Green tea composition, consumption, and polyphenol chemistry. *Prev Med, 21*(3), 334-350.

Granado, M., Priego, T., Martin, A. I., Villanua, M. A., and Lopez-Calderon, A. (2005). Anti-inflammatory effect of the ghrelin agonist growth hormone-releasing peptide-2 (GHRP-2) in arthritic rats. *Am J Physiol Endocrinol Metab, 288*(3), E486-492.

Grassi, F., Tell, G., Robbie-Ryan, M., Gao, Y., Terauchi, M., Yang, X., Romanello, M., Jones, D. P., Weitzmann, M. N., Pacifici, R. (2007). Oxidative stress causes bone loss in estrogen-deficient mice through enhanced bone marrow dendritic cell activation. *Proc Natl Acad Sci U S A, 104*(38), 15087-15092.

Greenberg, J. A., Axen, K. V., Schnoll, R., and Boozer, C. N. (2005). Coffee, tea and diabetes: the role of weight loss and caffeine. In*t J Obes (Lond), 29*(9), 1121-1129.

Greenberg, J. A., Boozer, C. N., and Geliebter, A. (2006). Coffee, diabetes, and weight control. *Am J Clin Nutr, 84*(4), 682-693.

Grover, J. K., Vats, V., and Yadav, S. S. (2005). Pterocarpus marsupium extract (Vijayasar) prevented the alteration in metabolic patterns induced in the normal rat by feeding an adequate diet containing fructose as sole carbohydrate. *Diabetes Obes Metab*, *7*(4), 414-420.

Guerre-Millo, M. (2002). Adipose tissue hormones. *J Endocrinol Invest, 25*(10), 855-861.

Guijarro, A., Laviano, A., and Meguid, M. M. (2006). Hypothalamic integration of immune function and metabolism. *Prog Brain Res, 153*, 367-405.

Gullu, I., and Marangoz, S. (1999). Induction of cachexia in mice. *Br J Cancer, 79*(9-10), 1620-1621.

Guo, P., Nishiyama, A., Rahman, M., Nagai, Y., Noma, T., Namba, T., Ishizawa, M., Murakami, K., Miyatake, A., Kimura, S., Mizushige, K., Abe, Y., Ohmori, K., Kohno, M. (2006). Contribution of reactive oxygen species to the pathogenesis of left ventricular failure in Dahl salt-sensitive hypertensive rats: effects of angiotensin II blockade. *J Hypertens, 24*(6), 1097-1104.

Ha, S. K., Park, H.S., Kim, S.J., Park, C.H., Kim, D.S., Kim, H.S. (1998). Prevalence and patterns of left ventricular hypertrophy in patients with predialysis chronic renal failure. *J Korean Med Sci. , 13*(5), 488-494.

Hales, C. N., and Barker, D. J. (2001). The thrifty phenotype hypothesis. *Br Med Bull, 60*, 5-20.

Haluzik, M., Parizkova, J., and Haluzik, M. M. (2004). Adiponectin and its role in the obesity-induced insulin resistance and related complications. *Physiol Res, 53*(2), 123-129.

Han, L. K., Takaku, T., Li, J., Kimura, Y., and Okuda, H. (1999). Anti-obesity action of oolong tea. *Int J Obes Relat Metab Disord, 23*(1), 98-105.

Handa, T., Yamaguchi, K., Sono, Y., and Yazawa, K. (2005). Effects of fenugreek seed extract in obese mice fed a high-fat diet. *Biosci Biotechnol Biochem, 69*(6), 1186-1188.

Harper, M. E. (1997). Obesity research continues to spring leaks. *Clin Invest Med, 20*(4), 239-244.

Harris, S. S., and Dawson-Hughes, B. (1994). Caffeine and bone loss in healthy

postmenopausal women. *Am J Clin Nutr, 60*(4), 573-578.

Hasegawa, G., Sawada, M., Nakamura, N., Nakano, K., and Kondo, M. (1991). The effects of overeating on insulin secretion in normal mice. *Diabetes Res, 17*(3), 139-145.

Hasegawa, N., Yamda, N., and Mori, M. (2003). Powdered green tea has antilipogenic effect on Zucker rats fed a high-fat diet. *Phytother Res, 17*(5), 477-480.

Hasnat, A. K., van der Velde, E. T., Hon, J. K., and Yacoub, M. H. (2003). Reproducible model of post-infarction left ventricular dysfunction: haemodynamic characterization by conductance catheter. *Eur J Cardiothorac Surg, 24*(1), 98-104.

Hattori, Y., Akimoto, K., Gross, S. S., Hattori, S., and Kasai, K. (2005). Angiotensin-II-induced oxidative stress elicits hypoadiponectinaemia in rats. *Diabetologia, 48*(6), 1066-1074.

Haugen, E. N., Croatt, A. J., and Nath, K. A. (2000). Angiotensin II induces renal oxidant stress in vivo and heme oxygenase-1 in vivo and in vitro. *Kidney Int, 58*(1), 144-152.

Hauner, H. (2005). Secretory factors from human adipose tissue and their functional role. *Proc Nutr Soc, 64*(2), 163-169.

Hausman, D. B., DiGirolamo, M., Bartness, T. J., Hausman, G. J., and Martin, R. J. (2001). The biology of white adipocyte proliferation. *Obes Rev, 2*(4), 239-254.

Hegarty, V. M., May, H. M., and Khaw, K. T. (2000). Tea drinking and bone mineral density in older women. *Am J Clin Nutr, 71*(4), 1003-1007.

Heilbronn, L., Smith, S. R., and Ravussin, E. (2004). Failure of fat cell proliferation, mitochondrial function and fat oxidation results in ectopic fat storage, insulin resistance and type II diabetes mellitus. In*t J Obes Relat Metab Disord, 28 Suppl 4*, S12-21.

Héliès-Toussaint C, M. C., Rasmusen C, Tabbi-Anneni I, Cynober L, Grynberg A. (2005). Aortic banding in rat as a model to investigate malnutrition associated with heart failure. *Am J Physiol Regul Integr Comp Physiol. 288*(5), R1325-1331.

Hervey, G. R. (1953). Determination of creatinine by the Jaffe reaction. *Nature, 171*(4364), 1125.

Higashiura, K., Ura, N., Takada, T., Li, Y., TorII, T., Togashi, N., Takada, M., Takizawa, H., Shimamoto, K. (2000). The effects of an Angiotensin-converting enzyme inhibitor and an Angiotensin II receptor antagonist on insulin resistance in fructose-fed rats. *Am J Hypertens, 13*(3), 290-297.

Hirai, K., Ishiko, O., and Tisdale, M. (1997). Mechanism of depletion of liver glycogen in cancer cachexia. *Biochem Biophys Res Commun, 241*(1), 49-52.

Hodgson, J. M., Devine, A., Burke, V., Dick, I. M., and Prince, R. L. (2008). Chocolate consumption and bone density in older women. *Am J Clin Nutr, 87*(1), 175-180.

Homma, T., Tsujinaka, T., Kido, Y., IIjima, S., Yano, M., Ebisui, C., Kan, K., Mori, T. (1993). Effect of platelet on protein degradation in rat skeletal muscle. *Eur Surg Res, 25*(6), 358-365.

Hosoda, K., Matsuda, J., Itoh, H., Son, C., Doi, K., Tanaka, T., Fukunaga, Y., Yamori, Y., Nakao, K. (1999). New members of uncoupling protein family implicated in energy metabolism. *Clin Exp Pharmacol Physiol, 26*(7), 561-562.

Howard, R. J., Stopps, T. P., Moe, G. W., Gotlieb, A., and Armstrong, P. W. (1988). Recovery from heart failure: structural and functional analysis in a canine model. *Can J Physiol Pharmacol, 66*(12), 1505-1512.

Hsu, T. F., Kusumoto, A., Abe, K., Hosoda, K., Kiso, Y., Wang, M. F., Yamamoto, S. (2006). Polyphenol-enriched oolong tea increases fecal lipid excretion. *Eur J Clin*

Nutr, 60(11), 1330-1336.

Huber, J. T., and Gullion, J. S. (2003). Complementary and alternative medicine as represented in the HIV/AIDS body of knowledge: a bibliometric analysis. *Med Ref Serv Q, 22*(3), 23-32.

Huentelman, M. J., Grobe, J. L., Vazquez, J., Stewart, J. M., Mecca, A. P., Katovich, M. J., Ferrario, C.M., Raizada, M.K. (2005). Protection from Angiotensin II-induced cardiac hypertrophy and fibrosis by systemic lentiviral delivery of ACE2 in rats. *Exp Physiol, 90*(5), 783-790.

Hussain, T. (2003). Renal Angiotensin II receptors, hyperinsulinemia, and obesity. *Clin Exp Hypertens, 25*(7), 395-403.

Hussey, H. J., and Tisdale, M. J. (2000). Effect of the specific cyclooxygenase-2 inhibitor meloxicam on tumour growth and cachexia in a murine model. In*t J Cancer, 87*(1), 95-100.

Ikeda, I., Tsuda, K., Suzuki, Y., Kobayashi, M., Unno, T., Tomoyori, H., Goto, H., Kawata, Y., Imaizumi, K., Nozawa, A., Kakuda, T. (2005). Tea catechins with a galloyl moiety suppress postprandial hypertriacylglycerolemia by delaying lymphatic transport of dietary fat in rats. *J Nutr, 135*(2), 155-159.

Ikemoto, S., Takahashi, M., Tsunoda, N., Maruyama, K., Itakura, H., and Ezaki, O. (1996). High-fat diet-induced hyperglycemia and obesity in mice: differential effects of dietary oils. *Metabolism, 45*(12), 1539-1546.

Ikeuchi, M., Yamaguchi, K., Koyama, T., Sono, Y., and Yazawa, K. (2006). Effects of fenugreek seeds (Trigonella foenum greaecum) extract on endurance capacity in mice. *J Nutr Sci Vitaminol (Tokyo), 52*(4), 287-292.

Ingle, L., Rigby, A. S., Carroll, S., Butterly, R., King, R. F., Cooke, C. B., Cleland, J.G., Clark, A.L. (2007). Changes in body composition in patients with left ventricular

systolic dysfunction initiated on beta-blocker therapy. *Exp Clin Cardiol, 12*(1), 46-47.

Inui, A. (1999). Cancer anorexia-cachexia syndrome: are neuropeptides the key? *Cancer Res, 59*(18), 4493-4501.

Inui, A., and Meguid, M. M. (2003). Cachexia and obesity: two sides of one coin? *Curr Opin Clin Nutr Metab Care, 6*(4), 395-399.

Jackson, M. J. (2008). Free radicals generated by contracting muscle: by-products of metabolism or key regulators of muscle function? *Free Radic Biol Med, 44*(2), 132-141.

Jensen, M. D., & Haymond, M. W. (1991). Protein metabolism in obesity: effects of body fat distribution and hyperinsulinemia on leucine turnover. *Am J Clin Nutr, 53*(1), 172-176.

Jorgensen, E. A., Knigge, U., Warberg, J., & Kjaer, A. (2007). Histamine and the regulation of body weight. *Neuroendocrinology, 86*(3), 210-214.

Juhel, C., Armand, M., Pafumi, Y., Rosier, C., Vandermander, J., and Lairon, D. (2000). Green tea extract (AR25) inhibits lipolysis of triglycerides in gastric and duodenal medium in vitro. *J Nutr Biochem, 11*(1), 45-51.

Jungas, R. L., Halperin, M. L., and Brosnan, J. T. (1992). Quantitative analysis of amino acid oxidation and related gluconeogenesis in humans. *Physiol Rev, 72*(2), 419-448.

Kalantar-Zadeh-Zadeh, K., Horwich, T. B., Oreopoulos, A., Kovesdy, C. P., Younessi, H., Anker, S. D., Morley, J. E. (2007). Risk factor paradox in wasting diseases. *Curr Opin Clin Nutr Metab Care, 10*(4), 433-442.

Kalra, S. P., Dube, M. G., Pu, S., Xu, B., Horvath, T. L., & Kalra, P. S. (1999). Interacting appetite-regulating pathways in the hypothalamic regulation of body weight. *Endocr Rev, 20*(1), 68-100.

failure. An update beyond theoretical perspectives. *Heart Fail Rev, 11*(1), 65-74.

Kao, Y. H., Hiipakka, R. A., and Liao, S. (2000). Modulation of obesity by a green tea catechin. *Am J Clin Nutr, 72*(5), 1232-1234.

Katz, J. R., Taylor, N. F., Goodrick, S., Perry, L., Yudkin, J. S., and Coppack, S. W. (2000). Central obesity, depression and the hypothalamo-pituitary-adrenal axis in men and postmenopausal women. In*t J Obes Relat Metab Disord, 24*(2), 246-251.

Katzman, M. A., Jacobs, L., Marcus, M., Vermani, M., and Logan, A. C. (2007). Weight gain and psychiatric treatment: is there a role for green tea and conjugated linoleic acid? *Lipids Health Dis, 6*, 14.

Kennaway, E. L. (1921). The Estimation of Non-Protein Nitrogen in Blood by a Micro-Kjeldahl Method. *Biochem J, 15*(4), 510-512.

Kim, C. H., Kim, M. S., Youn, J. Y., Park, H. S., Song, H. S., Song, K. H., Park, J.Y., Lee, K.U. (2003). Lipolysis in skeletal muscle is decreased in high-fat-fed rats. *Metabolism, 52*(12), 1586-1592.

King, D., Smith, M. L., Chapman, T. J., Stockdale, H. R., and Lye, M. (1996). Fat malabsorption in elderly patients with cardiac cachexia. *Age Ageing, 25*(2), 144-149.

King, D., Smith, M. L., and Lye, M. (1996). Gastro-intestinal protein loss in elderly patients with cardiac cachexia. *Age Ageing, 25*(3), 221-223.

Klaus, S., Pultz, S., Thone-Reineke, C., and Wolfram, S. (2005). Epigallocatechin gallate attenuates diet-induced obesity in mice by decreasing energy absorption and increasing fat oxidation. In*t J Obes (Lond), 29*(6), 615-623.

Kobayashi-Hattori, K., Mogi, A., Matsumoto, Y., and Takita, T. (2005). Effect of caffeine on the body fat and lipid metabolism of rats fed on a high-fat diet. *Biosci Biotechnol Biochem, 69*(11), 2219-2223.

Komatsu, T., Nakamori, M., Komatsu, K., Hosoda, K., Okamura, M., Toyama, K.,

Ishikura, Y., Sakai, T., Kunii, D., Yamamoto, S. (2003). Oolong tea increases energy metabolism in Japanese females. *J Med Invest, 50*(3-4), 170-175.

Konturek, S. J., Konturek, J. W., Pawlik, T., and Brzozowski, T. (2004). Brain-gut axis and its role in the control of food intake. *J Physiol Pharmacol, 55*(1 Pt 2), 137-154.

Korstjens, I. J., Rouws, C. H., van der Laarse, W. J., Van der Zee, L., and Stienen, G. J. (2002). Myocardial force development and structural changes associated with monocrotaline induced cardiac hypertrophy and heart failure. *J Muscle Res Cell Motil, 23*(1), 93-102.

Kotler, D. P. (2000). Cachexia. *Ann Intern Med, 133*(8), 622-634.

Kozak, L. P., & Harper, M. E. (2000). Mitochondrial uncoupling proteins in energy expenditure. *Annu Rev Nutr, 20*, 339-363.

Krack, A., Sharma, R., Figulla, H. R., & Anker, S. D. (2005). The importance of the gastrointestinal system in the pathogenesis of heart failure. *Eur Heart J, 26*(22), 2368-2374.

Kraemer F. B., Shen W. J. (2002). Hormone-sensitive lipase: control of intracellular tri-(di-)acylglycerol and cholesteryl ester hydrolysis. *J. Lipid Res. 43(10)*, 1585–94.

Kramer, H. (2006). Obesity and chronic kidney disease. *Contrib Nephrol, 151*, 1-18.

Kumar, S., Kishimoto, H., Chua, H. L., Badve, S., Miller, K. D., Bigsby, R. M.,Nakshatri, H. (2003). Interleukin-1 alpha promotes tumour growth and cachexia in MCF-7 xenograft model of breast cancer. *Am J Pathol, 163*(6), 2531-2541.

Kuroda, K., Nakashima, J., Kanao, K., Kikuchi, E., Miyajima, A., Horiguchi, Y, Nakagawa, K., Oya, M., Ohigashi, T., Murai, M. (2007). Interleukin 6 is associated with cachexia in patients with prostate cancer. *Urology, 69*(1), 113-117.

Lainscak, M., Keber, I., and Anker, S. D. (2006). Body composition changes in

patients with systolic heart failure treated with beta blockers: a pilot study. In*t J Cardiol, 106*(3), 319-322.

Lam, R. Y., Woo, A. Y., Leung, P. S., and Cheng, C. H. (2007). Antioxidant actions of phenolic compounds found in dietary plants on low-density lipoprotein and erythrocytes in vitro. *J Am Coll Nutr, 26*(3), 233-242.

Lamson, D. W., and Brignall, M. S. (2001). Natural agents in the prevention of cancer, part two: preclinical data and chemoprevention for common cancers. *Altern Med Rev, 6*(2), 167-187.

Lange, K. H., Isaksson, F., Juul, A., Rasmussen, M. H., Bulow, J., and Kjaer, M. (2000). Growth hormone enhances effects of endurance training on oxidative muscle metabolism in elderly women. *Am J Physiol Endocrinol Metab, 279*(5), E989-996.

Langley-Evans, S. C. (2000). Antioxidant potential of green and black tea determined using the ferric reducing power (FRAP) assay. In*t J Food Sci Nutr, 51*(3), 181-188.

Leichman, J. G., Aguilar, D., King, T. M., Mehta, S., Majka, C., Scarborough, T. Wilson, E.B., Taegtmeyer, H. (2006). Improvements in systemic metabolism, anthropometrics, and left ventricular geometry 3 months after bariatric surgery. *Surg Obes Relat Dis, 2*(6), 592-599.

Leifert, W. R., Dorian, C. L., Jahangiri, A., and McMurchie, E. J. (2001). Dietary fish oil prevents asynchronous contractility and alters Ca(2+) handling in adult rat cardiomyocytes. *J Nutr Biochem, 12*(6), 365-376.

Leifert, W. R., Jahangiri, A., Saint, D. A., and McMurchie, E. J. (2000). Effects of dietary n-3 fatty acids on contractility, Na+ and K+ currents in a rat cardiomyocyte model of arrhythmia. *J Nutr Biochem, 11*(7-8), 382-392.

Leiter, L. A., and Lewanczuk, R. Z. (2005). Of the renin-Angiotensin system and reactive oxygen species Type 2 diabetes and Angiotensin II inhibition. *Am J*

Hypertens, 18(1), 121-128.

Levett, J. M., Marinelli, C. C., Lund, D. D., Pardini, B. J., Nader, S., Scott, B. D., Augelli, N.V., Kerber, R.E., Schmid, P.G. Jr. (1994). Effects of beta-blockade on neurohumoral responses and neurochemical markers in pacing-induced heart failure. *Am J Physiol, 266*(2 Pt 2), H468-475.

Littarru, G. P., and Tiano, L. (2007). Bioenergetic and antioxidant properties of coenzyme Q10: recent developments. *Mol Biotechnol, 37*(1), 31-37.

Little, R.A. (1991). 'Metabolic rate and its control after accidental injury', in Stock, M.J., and Rothwell, N.J. (1991). 'Obesity and Cachexia: Physiological Mechanisms and New Approaches to Pharmacological Control'. John Wiley and Sons Publishing, Chichester. U.K

Lommi, J., Kupari, M., and Yki-Jarvinen, H. (1998). Free fatty acid kinetics and oxidation in congestive heart failure. *Am J Cardiol, 81*(1), 45-50.

Lopez-Garcia, E., van Dam, R. M., Rajpathak, S., Willett, W. C., Manson, J. E., and Hu, F. B. (2006). Changes in caffeine intake and long-term weight change in men and women. *Am J Clin Nutr, 83*(3), 674-680.

Lorite, M. J., Thompson, M. G., Drake, J. L., Carling, G., and Tisdale, M. J. (1998). Mechanism of muscle protein degradation induced by a cancer cachectic factor. *Br J Cancer, 78*(7), 850-856.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *J Biol Chem, 193*(1), 265-275.

Lu, K., Gray, M. A., Oliver, C., Liley, D. T., Harrison, B. J., Bartholomeusz, C. F. Phan, K.L., Nathan, P.J. (2004). The acute effects of L-theanine in comparison with alprazolam on anticipatory anxiety in humans. *Hum Psychopharmacol, 19*(7), 457-465.

Lundholm, K., Korner, U., Gunnebo, L., Sixt-Ammilon, P., Fouladiun, M., Daneryd, P.,Bosaeus, I. (2007). Insulin treatment in cancer cachexia: effects on survival, metabolism, and physical functioning. *Clin Cancer Res, 13*(9), 2699-2706.

Lutz, E. G. (1978). Restless legs, anxiety and caffeinism. *J Clin Psychiatry, 39*(9), 693-698.

MacLeod, M. G. (1997). Effects of amino acid balance and energy:protein ratio on energy and nitrogen metabolism in male broiler chickens. *Br Poult Sci, 38*(4), 405-411.

Madar, Z. (1987). New sources of dietary fibre. In*t J Obes, 11 Suppl 1*, 57-65.

Majalahti, T., Suo-Palosaari, M., Sarman, B., Hautala, N., Pikkarainen, S., Tokola, H., Vuolteenaho, O., Wang, J., Paradis, P., Nemer, M., Ruskoaho, H. (2007). Cardiac BNP gene activation by Angiotensin II in vivo. *Mol Cell Endocrinol, 273*(1-2), 59-67.

Mak, R.H., and Cheung, W.W. (2007). 'Nutrition and Health Book - Adipose Tissue and Adipokines in Health and Disease' Chapter 19 - Mechanisms of cachexia. p255-264 Publisher Humana Press

Mann, D. L., and Young, J. B. (1994). Basic mechanisms in congestive heart failure. Recognizing the role of proinflammatory cytokines. *Chest, 105*(3), 897-904.

Mantena, S. K., King, A. L., Andringa, K. K., Eccleston, H. B., and Bailey, S. M. (2008). Mitochondrial dysfunction and oxidative stress in the pathogenesis of alcohol- and obesity-induced fatty liver diseases. *Free Radic Biol Med, 44*(7), 1259-1272.

Mantovani, G., Madeddu, C., Maccio, A., Gramignano, G., Lusso, M. R., Massa, E., Astara, G., Serpe, R. (2004). Cancer-related anorexia/cachexia syndrome and oxidative stress: an innovative approach beyond current treatment. *Cancer Epidemiol Biomarkers Prev, 13*(10), 1651-1659.

Marcelli, E., Cercenelli, L., Parlapiano, M., Fumero, R., Bagnoli, P., Costantino, M.

L., Plicchi, G. (2007). Effect of right ventricular pacing on cardiac apex rotation assessed by a gyroscopic sensor. *Asaio J, 53*(3), 304-309.

Matsui, N., Ito, R., Nishimura, E., Yoshikawa, M., Kato, M., Kamei, M., Shibata, H., Matsumoto, I., Abe, K., Hashizume, S. (2005). Ingested cocoa can prevent high-fat diet-induced obesity by regulating the expression of genes for fatty acid metabolism. *Nutrition, 21*(5), 594-601.

McCarthy, D. O. (1999). Inhibitors of prostaglandin synthesis do not improve food intake or body weight of tumour-bearing rats. *Res Nurs Health, 22*(5), 380-387.

McCarthy, D. O., Lo, C., Nguyen, H., and Ney, D. M. (1997). The effect of protein density of food on food intake and nutritional status of tumour-bearing rats. *Res Nurs Health, 20*(2), 131-138.

McDevitt, T. M., Todorov, P. T., Beck, S. A., Khan, S. H., and Tisdale, M. J. (1995). Purification and characterization of a lipid-mobilizing factor associated with cachexia-inducing tumours in mice and humans. *Cancer Res, 55*(7), 1458-1463.

McPherron, A. C., and Lee, S. J. (1997). Double muscling in cattle due to mutations in the myostatin gene. *Proc Natl Acad Sci U S A, 94*(23), 12457-12461.

Meadows, K. A., Holly, J. M., and Stewart, C. E. (2000). Tumor necrosis factor-alpha-induced apoptosis is associated with suppression of insulin-like growth factor binding protein-5 secretion in differentiating murine skeletal myoblasts. *J Cell Physiol, 183*(3), 330-337.

Merial, C., Bouloumie, A., Trocheris, V., Lafontan, M., and Galitzky, J. (2000). Nitric oxide-dependent downregulation of adipocyte UCP-2 expression by tumour necrosis factor-alpha. *Am J Physiol Cell Physiol, 279*(4), C1100-1106.

Middleton, J. E., and Griffiths, W. J. (1957). Rapid colorimetric micro-method for estimating glucose in blood and C. S. F. using glucose oxidase. *Br Med J, 2*(5060),

1525-1527.

Millis, R. M., Diya, C. A., Reynolds, M. E., Dehkordi, O., and Bond, V., Jr. (1998). Growth inhibition of subcutaneously transplanted hepatomas without cachexia by alteration of the dietary arginine-methionine balance. *Nutr Cancer, 31*(1), 49-55.

Miura, T., Koike, T., Ishida, T. . (2005). Antidiabetic activity of green tea (Thea sinensis L.) in genetically type 2 diabetic mice. . *Journal of Health Science 51*(6), 708-710.

Mochizuki, M., and Hasegawa, N. (2004). Effects of green tea catechin-induced lipolysis on cytosol glycerol content in differentiated 3T3-L1 cells. *Phytother Res, 18*(11), 945-946.

Moe, G. W., and Armstrong, P. (1999). Pacing-induced heart failure: a model to study the mechanism of disease progression and novel therapy in heart failure. *Cardiovasc Res, 42*(3), 591-599.

Mohammad, S., Taha, A., Akhtar, K., Bamezai, R. N., and Baquer, N. Z. (2006). In vivo effect of Trigonella foenum graecum on the expression of pyruvate kinase, phosphoenolpyruvate carboxykinase, and distribution of glucose transporter (GLUT4) in alloxan-diabetic rats. *Can J Physiol Pharmacol, 84*(6), 647-654.

Montgomery, C., Hamilton, N., and Ianuzzo, C. D. (1992). Energy status of the rapidly paced canine myocardium in congestive heart failure. *J Appl Physiol, 73*(6), 2363-2367.

Moore, S., and Stein, W. H. (1954). A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. *J Biol Chem, 211*(2), 907-913.

Mori, T., Kondo, H., Hase, T., Tokimitsu, I., and Murase, T. (2007). Dietary fish oil upregulates intestinal lipid metabolism and reduces body weight gain in C57BL/6J mice. *J Nutr, 137*(12), 2629-2634.

Morley, J. E., Thomas, D. R., & Wilson, M. M. (2006). Cachexia: pathophysiology and clinical relevance. *Am J Clin Nutr, 83*(4), 735-743.

Morrison, W. L., Gibson, J. N., and Rennie, M. J. (1988). Skeletal muscle and whole body protein turnover in cardiac cachexia: influence of branched-chain amino acid administration. *Eur J Clin Invest, 18*(6), 648-654.

Mougios, V., Ring, S., Petridou, A., and Nikolaidis, M. G. (2003). Duration of coffee- and exercise-induced changes in the fatty acid profile of human serum. *J Appl Physiol, 94*(2), 476-484.

Muraki, S., Yamamoto, S., Ishibashi, H., Oka, H., Yoshimura, N., Kawaguchi, H., Nakamura, K. (2007). Diet and lifestyle associated with increased bone mineral density: cross-sectional study of Japanese elderly women at an osteoporosis outpatient clinic. *J Orthop Sci, 12*(4), 317-320.

Murase, T., Haramizu, S., Shimotoyodome, A., Nagasawa, A., and Tokimitsu, I. (2005). Green tea extract improves endurance capacity and increases muscle lipid oxidation in mice. *Am J Physiol Regul Integr Comp Physiol, 288*(3), R708-715.

Murase, T., Haramizu, S., Shimotoyodome, A., and Tokimitsu, I. (2006). Reduction of diet-induced obesity by a combination of tea-catechin intake and regular swimming. In*t J Obes (Lond), 30*(3), 561-568.

Murase, T., Haramizu, S., Shimotoyodome, A., Tokimitsu, I., and Hase, T. (2006). Green tea extract improves running endurance in mice by stimulating lipid utilization during exercise. *Am J Physiol Regul integr Comp Physiol, 290*(6), R1550-1556.

Murase, T., Nagasawa, A., Suzuki, J., Hase, T., and Tokimitsu, I. (2002). Beneficial effects of tea catechins on diet-induced obesity: stimulation of lipid catabolism in the liver. In*t J Obes Relat Metab Disord, 26*(11), 1459-1464.

Murray, A. J., Edwards, L. M., and Clarke, K. (2007). Mitochondria and heart failure.

Curr Opin Clin Nutr Metab Care, 10(6), 704-711.

Muscaritoli, M., Bossola, M., Aversa, Z., Bellantone, R., and Rossi Fanelli, F. (2006). Prevention and treatment of cancer cachexia: new insights into an old problem. *Eur J Cancer, 42*(1), 31-41.

Mustafa, I., and Leverve, X. (2001). Metabolic and nutritional disorders in cardiac cachexia. *Nutrition, 17*(9), 756-760.

Nagao, T., Hase, T., and Tokimitsu, I. (2007). A green tea extract high in catechins reduces body fat and cardiovascular risks in humans. *Obesity (Silver Spring), 15*(6), 1473-1483.

Nagao, T., Komine, Y., Soga, S., Meguro, S., Hase, T., Tanaka, Y.,Tokimitsu, I. (2005). Ingestion of a tea rich in catechins leads to a reduction in body fat and malondialdehyde-modified LDL in men. *Am J Clin Nutr, 81*(1), 122-129.

Nagaya, N., Kojima, M., and Kangawa, K. (2006). Ghrelin, a novel growth hormone-releasing peptide, in the treatment of cardiopulmonary-associated cachexia. In*tern Med, 45*(3), 127-134.

Nagaya, N., Uematsu, M., Kojima, M., Date, Y., Nakazato, M., Okumura, H., Hosoda, H., Shimizu, W., Yamagishi, M., Oya, H., Koh, H., Yutani, C., Kangawa, K.. (2001). Elevated circulating level of ghrelin in cachexia associated with chronic heart failure: relationships between ghrelin and anabolic/catabolic factors. *Circulation, 104*(17), 2034-2038.

Nagaya, N., Uematsu, M., Kojima, M., Ikeda, Y., Yoshihara, F., Shimizu, W., Hosoda, H., Hirota, Y., Ishida, H., Mori, H., Kangawa, K. (2001). Chronic administration of ghrelin improves left ventricular dysfunction and attenuates development of cardiac cachexia in rats with heart failure. *Circulation, 104*(12), 1430-1435.

Negre-Salvayre, A., Hirtz, C., Carrera, G., Cazenave, R., Troly, M., Salvayre, R.,Pénicaud, L., Casteilla, L. (1997). A role for uncoupling protein-2 as a regulator of mitochondrial hydrogen peroxide generation. *Faseb J, 11*(10), 809-815.

Neumann, T., Vollmer, A., Schaffner, T., Hess, O. M., and Heusch, G. (1999). Diastolic dysfunction and collagen structure in canine pacing-induced heart failure. *J Mol Cell Cardiol, 31*(1), 179-192.

Nicklas, B. J., Cesari, M., Penninx, B. W., Kritchevsky, S. B., Ding, J., Newman, A., Kitzman, D.W., Kanaya, A.M., Pahor, M., Harris, T.B. (2006). Abdominal obesity is an independent risk factor for chronic heart failure in older people. *J Am Geriatr Soc, 54*(3), 413-420.

Nixon, D. W., Heymsfield, S. B., Cohen, A. E., Kutner, M. H., Ansley, J., Lawson, D. H., Rudman, D. (1980). Protein-calorie undernutrition in hospitalized cancer patients. *Am J Med, 68*(5), 683-690.

Nordfors, L., Hoffstedt, J., Nyberg, B., Thorne, A., Arner, P., Schalling, M., Lönnqvist, F. (1998). Reduced gene expression of UCP2 but not UCP3 in skeletal muscle of human obese subjects. *Diabetologia, 41*(8), 935-939.

Norrelund, H., Wiggers, H., Halbirk, M., Frystyk, J., Flyvbjerg, A., Botker, H. E., Schmitz, O., Jorgensen, J. O., Christiansen, J. S., & Moller, N. (2006). Abnormalities of whole body protein turnover, muscle metabolism and levels of metabolic hormones in patients with chronic heart failure. *J Intern Med, 260*(1), 11-21.

Norton, J. A., Peacock, J. L., and Morrison, S. D. (1987). Cancer cachexia. *Crit Rev Oncol Hematol, 7*(4), 289-327.

Oldenburg, H. S., Rogy, M. A., Lazarus, D. D., Van Zee, K. J., Keeler, B. P., Chizzonite, R. A., Lowry, S.F., Moldawer, L.L. (1993). Cachexia and the acute-phase protein response in inflammation are regulated by interleukin-6. *Eur J Immunol,*

23(8), 1889-1894.

Otaki, M. (1994). Surgical treatment of patients with cardiac cachexia. An analysis of factors affecting operative mortality. *Chest, 105*(5), 1347-1351.

Palmieri, V., Roman, M. J., Bella, J. N., Liu, J. E., Best, L. G., Lee, E. T., Howard, B.V., Devereux, R.B. (2008). Prognostic implications of relations of left ventricular systolic dysfunction with body composition and myocardial energy expenditure: the Strong Heart Study. *J Am Soc Echocardiogr, 21*(1), 66-71.

Pan, J. P., Liu, T. Y., Chiang, S. C., Lin, Y. K., Chou, C. Y., Chan, W. L., Lai, S.T. (2004). The value of plasma levels of tumour necrosis factor-alpha and interleukin-6 in predicting the severity and prognosis in patients with congestive heart failure. *J Chin Med Assoc, 67*(5), 222-228.

Panda, S., Tahiliani, P., and Kar, A. (1999). Inhibition of triiodothyronine production by fenugreek seed extract in mice and rats. *Pharmacol Res, 40*(5), 405-409.

Park, J. Y., Park, K. G., Kim, H. J., Kang, H. G., Ahn, J. D., Kim, H. S.,Kim, Y.M., Son, S.M., Kim, I.J., Kim, Y.K., Kim, C.D., Lee, K.U., Lee, I.K. (2005). The effects of the overexpression of recombinant uncoupling protein 2 on proliferation, migration and plasminogen activator inhibitor 1 expression in human vascular smooth muscle cells. *Diabetologia, 48*(5), 1022-1028.

Parra, D., Bandarra, N. M., Kiely, M., Thorsdottir, I., and Martinez, J. A. (2007). impact of fish intake on oxidative stress when included into a moderate energy-restricted program to treat obesity. *Eur J Nutr, 46*(8), 460-467.

Pasini, E., Aquilani, R., Gheorghiade, M., & Dioguardi, F. S. (2003). Malnutrition, muscle wasting and cachexia in chronic heart failure: the nutritional approach. *Ital Heart J, 4*(4), 232-235.

Patiag, D., Qu, X., Gray, S., Idris, I., Wilkes, M., Seale, J. P., Donnelly, R. (2000).

Possible interactions between Angiotensin II and insulin: effects on glucose and lipid metabolism in vivo and in vitro. *J Endocrinol, 167*(3), 525-531.

Paulus, W. J. (2000). Cytokines and heart failure. *Heart Fail Monit, 1*(2), 50-56.

Pecqueur, C., Alves-Guerra, M. C., Gelly, C., Levi-Meyrueis, C., Couplan, E., Collins, S., Ricquier, D., Bouillaud, F., Miroux, B. (2001). Uncoupling protein 2, in vivo distribution, induction upon oxidative stress, and evidence for translational regulation. *J Biol Chem, 276*(12), 8705-8712.

Penfornis, P., and Marette, A. (2005). Inducible nitric oxide synthase modulates lipolysis in adipocytes. *J Lipid Res, 46*(1), 135-142.

Perusse, L., and Bouchard, C. (2000). Gene-diet interactions in obesity. *Am J Clin Nutr, 72*(5 Suppl), 1285S-1290S.

Peterson, J. M., Feeback, K. D., Baas, J. H., and Pizza, F. X. (2006). Tumor necrosis factor-alpha promotes the accumulation of neutrophils and macrophages in skeletal muscle. *J Appl Physiol, 101*(5), 1394-1399.

Piepoli, M. F., Kaczmarek, A., Francis, D. P., Davies, L. C., Rauchhaus, M., Jankowska, E. A., Anker SD, Capucci A, Banasiak W, Ponikowski P. (2006). Reduced peripheral skeletal muscle mass and abnormal reflex physiology in chronic heart failure. *Circulation, 114*(2), 126-134.

Pinnell, A. E., and Northam, B. E. (1978). New automated dye-binding method for serum albumin determination with bromcresol purple. *Clin Chem, 24*(1), 80-86.

Pinterova, L., Zelezna, B., Fickova, M., Macho, L., Krizanova, O., Jezova, D., Zórad, S. (2001). Elevated AT1 receptor protein but lower Angiotensin II-binding in adipose tissue of rats with monosodium glutamate-induced obesity. *Horm Metab Res, 33*(12), 708-712.

Pi-Sunyer, F. X. (2000). Overnutrition and undernutrition as modifiers of metabolic

processes in disease states. *Am J Clin Nutr, 72*(2 Suppl), 533S-537S.

Poehlman, E. T. (1999). Special considerations in design of trials with elderly subjects: unexplained weight loss, body composition and energy expenditure. *J Nutr, 129*(1S Suppl), 260S-263S.

Poehlman, E. T., Scheffers, J., Gottlieb, S. S., Fisher, M. L., and Vaitekevicius, P. (1994). Increased resting metabolic rate in patients with congestive heart failure. *Ann Intern Med, 121*(11), 860-862.

Poehlman, E. T., Toth, M. J., Fishman, P. S., Vaitkevicius, P., Gottlieb, S. S., Fisher, M. L., Fonong, T. (1995). Sarcopenia in aging humans: the impact of menopause and disease. *J Gerontol A Biol Sci Med Sci, 50 Spec No*, 73-77.

Porter, J. P. (1999). Chronic intracerebroventricular infusion of Angiotensin II increases brain AT1 receptor expression in young rats. *Brain Res Dev Brain Res, 112*(2), 293-295.

Porter, J. P., Anderson, J. M., Robison, R. J., and Phillips, A. C. (2003). Effect of central Angiotensin II on body weight gain in young rats. *Brain Res, 959*(1), 20-28.

Porter, J. P., and Potratz, K. R. (2004). Effect of intracerebroventricular Angiotensin II on body weight and food intake in adult rats. *Am J Physiol Regul Integr Comp Physiol, 287*(2), R422-428.

Porter, R. K., and Brand, M. D. (1993). Body mass dependence of H+ leak in mitochondria and its relevance to metabolic rate. *Nature, 362*(6421), 628-630.

Power, J. M., and Tonkin, A. M. (1999). Large animal models of heart failure. *Aust N Z J Med, 29*(3), 395-402.

Preumont, N., Jansens, J. L., Berkenboom, G., van de Borne, P., Stoupel, E., and Goldman, S. (2005). Effects of right ventricular pacing on regional myocardial glucose metabolism. *Europace, 7*(6), 584-591.

Prima, V., Tennant, M., Gorbatyuk, O. S., Muzyczka, N., Scarpace, P. J., and Zolotukhin, S. (2004). Differential modulation of energy balance by leptin, ciliary neurotrophic factor, and leukemia inhibitory factor gene delivery: microarray deoxyribonucleic acid-chip analysis of gene expression. *Endocrinology, 145*(4), 2035-2045.

Puri, D., Prabhu, K. M., and Murthy, P. S. (2002). Mechanism of action of a hypoglycemic principle isolated from fenugreek seeds. In*dian J Physiol Pharmacol, 46*(4), 457-462.

Rademaker, M. T., Charles, C. J., Lewis, L. K., Yandle, T. G., Cooper, G. J., Coy, D. H., Richards, A.M., Nicholls, M.G. (1997). Beneficial hemodynamic and renal effects of adrenomedullin in an ovine model of heart failure. *Circulation, 96*(6), 1983-1990.

Rademaker, M. T., Charles, C. J., and Richards, A. M. (2007). Urocortin 1 administration from onset of rapid left ventricular pacing represses progression to overt heart failure. *Am J Physiol Heart Circ Physiol, 293*(3), H1536-1544.

Raimbault, S., Dridi, S., Denjean, F., Lachuer, J., Couplan, E., Bouillaud, F., Bordas, A., Duchamp, C., Taouis, M., Ricquier, D. (2001). An uncoupling protein homologue putatively involved in facultative muscle thermogenesis in birds. *Biochem J, 353*(Pt 3), 441-444.

Raju, J., and Bird, R. P. (2006). Alleviation of hepatic steatosis accompanied by modulation of plasma and liver TNF-alpha levels by Trigonella foenum graecum (fenugreek) seeds in Zucker obese (fa/fa) rats. In*t J Obes (Lond), 30*(8), 1298-1307.

Ramsey, J. J., Johnson, D. E., Hossner, K. L., and Johnson, K. A. (1996). Metabolic rate, organ mass, and mitochondrial proton leak variations in lean and obese rats. *Comp Biochem Physiol B Biochem Mol Biol, 113*(3), 461-466.

Ran, J., Hirano, T., and Adachi, M. (2004). Chronic ANG II infusion increases plasma

triglyceride level by stimulating hepatic triglyceride production in rats. *Am J Physiol Endocrinol Metab, 287*(5), E955-961.

Raneva, V. G., and Shimasaki, H. . (2005). Green Tea Catechins Decrease Lipid Peroxidation in Plasma and Organs of C57BL/6J Mice Fed Atherogenic Diet *Journal of Oleo Science 54*(12), 641-648.

Rapuri, P. B., Gallagher, J. C., Kinyamu, H. K., and Ryschon, K. L. (2001). Caffeine intake increases the rate of bone loss in elderly women and interacts with vitamin D receptor genotypes. *Am J Clin Nutr, 74*(5), 694-700.

Ravikumar, P., and Anuradha, C. V. (1999). Effect of fenugreek seeds on blood lipid peroxidation and antioxidants in diabetic rats. *Phytother Res, 13*(3), 197-201.

Rguibi, M., and Belahsen, R. (2006). Fattening practices among Moroccan Saharawi women. *East Mediterr Health J, 12*(5), 619-624.

Ricquier, D., and Bouillaud, F. (2000). Mitochondrial uncoupling proteins: from mitochondria to the regulation of energy balance. *J Physiol, 529 Pt 1*, 3-10.

Rihs, M., Muller, C., and Baumann, P. (1996). Caffeine consumption in hospitalized psychiatric patients. *Eur Arch Psychiatry Clin Neurosci, 246*(2), 83-92.

Rippe, C., Berger, K., Boiers, C., Ricquier, D., and Erlanson-Albertsson, C. (2000). Effect of high-fat diet, surrounding temperature, and enterostatin on uncoupling protein gene expression. *Am J Physiol Endocrinol Metab, 279*(2), E293-300.

Rist, C. B., Watts, J. C., and Lucas, R. J. (1984). Isolated Ischemic necrosis of the cecum in patients with chronic heart disease. *Dis Colon Rectum, 27*(8), 548-551.

Roberts, S. B., Fuss, P., Heyman, M. B., Evans, W. J., Tsay, R., Rasmussen, H., Fiatarone, M., Cortiella, J., Dallal, G.E., Young, V.R. (1994). Control of food intake in older men. *Jama, 272*(20), 1601-1606.

Rolfe, D. F., and Brown, G. C. (1997). Cellular energy utilization and molecular

origin of standard metabolic rate in mammals. *Physiol Rev, 77*(3), 731-758.

Rolfe, D. F., Newman, J. M., Buckingham, J. A., Clark, M. G., and Brand, M. D. (1999). Contribution of mitochondrial proton leak to respiration rate in working skeletal muscle and liver and to SMR. *Am J Physiol, 276*(3 Pt 1), C692-699.

Rolls, E. T., and McCabe, C. (2007). Enhanced affective brain representations of chocolate in cravers vs. non-cravers. *Eur J Neurosci, 26*(4), 1067-1076.

Rosenthal, N., and Musaro, A. (2002). Gene therapy for cardiac cachexia? *Int J Cardiol, 85*(1), 185-191.

Roubenoff, R. (2004). Sarcopenic obesity: the confluence of two epidemics. *Obes Res, 12*(6), 887-888.

Rousset, S., Alves-Guerra, M. C., Mozo, J., Miroux, B., Cassard-Doulcier, A. M., Bouillaud, F.,Ricquier, D. (2004). The biology of mitochondrial uncoupling proteins. *Diabetes, 53 Suppl 1*, S130-135.

Rubin, H. (2003). Cancer cachexia: its correlations and causes. *Proc Natl Acad Sci U S A, 100*(9), 5384-5389.

Rumpler, W., Seale, J., Clevidence, B., Judd, J., Wiley, E., Yamamoto, S., Komatsu, T., Sawaki, T., Ishikura, Y., Hosoda, K. (2001). Oolong tea increases metabolic rate and fat oxidation in men. *J Nutr, 131*(11), 2848-2852.

Russell, S. T., Hirai, K., and Tisdale, M. J. (2002). Role of beta3-adrenergic receptors in the action of a tumour lipid mobilizing factor. *Br J Cancer, 86*(3), 424-428.

Saarinen, U. M., Koskelo, E. K., Teppo, A. M., and SIImes, M. A. (1990). Tumor necrosis factor in children with malignancies. *Cancer Res, 50*(3), 592-595.

Samra, J. S. (2000). Sir David Cuthbertson Medal Lecture. Regulation of lipid metabolism in adipose tissue. *Proc Nutr Soc, 59*(3), 441-446.

Samuels, S. E., Knowles, A. L., Tilignac, T., Debiton, E., Madelmont, J. C., and

Attaix, D. (2001). Higher skeletal muscle protein synthesis and lower breakdown after chemotherapy in cachectic mice. *Am J Physiol Regul Integr Comp Physiol, 281*(1), R133-139.

Sanders, P. M., Tisdale, M.J. (2004). Role of lipid-mobilising factor (LMF) in protecting tumour cells from oxidative damage. *Br J Cancer, 90*(6), 1274-1278.

Savi, L., Rainero, I., Valfre, W., Gentile, S., Lo Giudice, R., and Pinessi, L. (2002). Food and headache attacks. A comparison of patients with migraine and tension-type headache. *Panminerva Med, 44*(1), 27-31.

Schein, P. S., Kisner, D., Haller, D., Blecher, M., and Hamosh, M. (1979). Cachexia of malignancy: potential role of insulin in nutritional management. *Cancer, 43*(5 Suppl), 2070-2076.

Schemmel, R., Hu, D., Mickelsen, O., & Romsos, D. R. (1982). Dietary obesity in rats: influence on carbohydrate metabolism. *J Nutr, 112*(2), 223-230.

Schulze, P. C., Gielen, S., Adams, V., Linke, A., Mobius-Winkler, S., Erbs, S., Kratzsch, J., Hambrecht, R., Schuler, G. (2003). Muscular levels of proinflammatory cytokines correlate with a reduced expression of insulinlike growth factor-I in chronic heart failure. *Basic Res Cardiol, 98*(4), 267-274.

Schulze, P. C., Kratzsch, J., Linke, A., Schoene, N., Adams, V., Gielen, S., Erbs, S., Moebius-Winkler, S., Schuler, G. (2003). Elevated serum levels of leptin and soluble leptin receptor in patients with advanced chronic heart failure. *Eur J Heart Fail, 5*(1), 33-40.

Schulze, P. C., and Spate, U. (2005). Insulin-like growth factor-1 and muscle wasting in chronic heart failure. In*t J Biochem Cell Biol, 37*(10), 2023-2035.

Schuman, M., Gitlin, M. J., and Fairbanks, L. (1987). Sweets, chocolate, and atypical depressive traits. *J Nerv Ment Dis, 175*(8), 491-495.

Scott, K. M., McGee, M. A., Wells, J. E., and Oakley Browne, M. A. (2008). Obesity and mental disorders in the adult general population. *J Psychosom Res, 64*(1), 97-105.

Seevaratnam, N., Bennett, A. J., Webber, J., and Macdonald, I. A. (2007). The effects of underfeeding on whole-body carbohydrate partitioning, thermogenesis and uncoupling protein 3 expression in human skeletal muscle. *Diabetes Obes Metab, 9*(5), 669-678.

Serisier, S., Leray, V., Poudroux, W., Magot, T., Ouguerram, K., and Nguyen, P. (2008). Effects of green tea on insulin sensitivity, lipid profile and expression of PPARalpha and PPARgamma and their target genes in obese dogs. *Br J Nutr, 99*(6), 1208-1216.

Seymour, E. M., Parikh, R. V., Singer, A. A., & Bolling, S. F. (2006). Moderate calorie restriction improves cardiac remodeling and diastolic dysfunction in the Dahl-SS rat. *J Mol Cell Cardiol, 41*(4), 661-668.

Sharma, A. M. (2004). Is there a rationale for Angiotensin blockade in the management of obesity hypertension? *Hypertension, 44*(1), 12-19.

Sharma, A. M., and Chetty, V. T. (2005). Obesity, hypertension and insulin resistance. *Acta Diabetol, 42 Suppl 1*, S3-8.

Sharma, A. M., Engeli, S., and Pischon, T. (2001). New developments in mechanisms of obesity-induced hypertension: role of adipose tissue. *Curr Hypertens Rep, 3*(2), 152-156.

Sharma, R., Al-Nasser, F. O., and Anker, S. D. (2001). The importance of tumour necrosis factor and lipoproteins in the pathogenesis of chronic heart failure. *Heart Fail Monit, 2*(2), 42-47.

Sharma, R. D., Raghuram, T. C., and Rao, N. S. (1990). Effect of fenugreek seeds on blood glucose and serum lipids in type I diabetes. *Eur J Clin Nutr, 44*(4), 301-306.

Sharma, V., and McNeill, J. H. (2005). The emerging roles of leptin and ghrelin in cardiovascular physiology and pathophysiology. *Curr Vasc Pharmacol, 3*(2), 169-180.

Shearer, J., Sellars, E. A., Farah, A., Graham, T. E., and Wasserman, D. H. (2007). Effects of chronic coffee consumption on glucose kinetics in the conscious rat. *Can J Physiol Pharmacol, 85*(8), 823-830.

Shen, C. L., Wang, P., Guerrieri, J., Yeh, J. K., and Wang, J. S. (2008). Protective effect of green tea polyphenols on bone loss in middle-aged female rats. *Osteoporos Int, 19*(7), 979-990.

Shimada, K., Kawarabayashi, T., Tanaka, A., Fukuda, D., Nakamura, Y., Yoshiyama, M., Takeuchi, K., Sawaki, T., Hosoda, K., Yoshikawa, J. (2004). Oolong tea increases plasma adiponectin levels and low-density lipoprotein particle size in patients with coronary artery disease. *Diabetes Res Clin Pract, 65*(3), 227-234.

Shimoda, H., Seki, E., and Aitani, M. (2006). Inhibitory effect of green coffee bean extract on fat accumulation and body weight gain in mice. *BMC Complement Altern Med, 6,* 9.

Shimotoyodome, A., Haramizu, S., Inaba, M., Murase, T., and Tokimitsu, I. (2005). Exercise and green tea extract stimulate fat oxidation and prevent obesity in mice. *Med Sci Sports Exerc, 37*(11), 1884-1892.

Silva, C. L., Tincani, I., Brandao Filho, S. L., and Faccioli, L. H. (1988). Mouse cachexia induced by trehalose dimycolate from Nocardia asteroides. *J Gen Microbiol, 134*(6), 1629-1633.

Silva, J. E. (1999). The physiological role of the novel uncoupling proteins: view from the chair. In*t J Obes Relat Metab Disord, 23 Suppl 6,* S72-74.

Silverberg, D. S., Wexler, D., Iaina, A., and Schwartz, D. (2006). The interaction

between heart failure and other heart diseases, renal failure, and anemia. *Semin Nephrol, 26*(4), 296-306.

Simons, J. P., Schols, A.M., Hoefnagels, J.M., Westerterp, K.R., ten Velde, G.P., Wouters, E.F. (1998). Effects of medroxyprogesterone acetate on food intake, body composition, and resting energy expenditure in patients with advanced, nonhormone-sensitive cancer: a randomized, placebo-controlled trial. *Cancer. , 82*(3), 553-560.

Skopinski, P., Skopinska-Rozewska, E., Sommer, E., Chorostowska-Wynimko, J., Rogala, E., Cendrowska, I., Chrystowska, D., Filewska, M., Białas-Chromiec., B, Bany, J. (2003). Chocolate feeding of pregnant mice influences length of limbs of their progeny. *Pol J Vet Sci, 6*(3 Suppl), 57-59.

Smith, K. L., and Tisdale, M. J. (1993). Mechanism of muscle protein degradation in cancer cachexia. *Br J Cancer, 68*(2), 314-318.

Soboll, S. (1995). Regulation of energy metabolism in liver. *J Bioenerg Biomembr, 27*(6), 571-582.

Song, Y. H., Li, Y., Du, J., Mitch, W. E., Rosenthal, N., and Delafontaine, P. (2005). Muscle-specific expression of IGF-I blocks Angiotensin II-induced skeletal muscle wasting. *J Clin Invest, 115*(2), 451-458.

Souza, C. G., Moreira, J. D., Siqueira, I. R., Pereira, A. G., Rieger, D. K., Souza, D. O., Souza, T.M., Portela, L.V., Perry, M.L. (2007). Highly palatable diet consumption increases protein oxidation in rat frontal cortex and anxiety-like behavior. *Life Sci, 81*(3), 198-203.

Spencer, J. P., Abd El Mohsen, M. M., Minihane, A. M., and Mathers, J. C. (2008). Biomarkers of the intake of dietary polyphenols: strengths, limitations and application in nutrition research. *Br J Nutr, 99*(1), 12-22.

Stadtman, E. R. (1992). Protein oxidation and aging. *Science, 257*(5074), 1220-1224.

Stark, A., and Madar, Z. (1993). The effect of an ethanol extract derived from fenugreek (Trigonella foenum-graecum) on bile acid absorption and cholesterol levels in rats. *Br J Nutr, 69*(1), 277-287.

Steffen, B. T., Lees, S. J., & Booth, F. W. (2008). Anti-TNF treatment reduces rat skeletal muscle wasting in monocrotaline-induced cardiac cachexia. *J Appl Physiol, 105*(6), 1950-1958.

Stuart, N. W. (1936). Adaptation of the Micro-Kjeldahl Method for the Determination of Nitrogen in Plant Tissues. *Plant Physiol, 11*(1), 173-179.

Stuart, J. A., Brindle, K. M., Harper, J. A., & Brand, M. D. (1999). Mitochondrial proton leak and the uncoupling proteins. *J Bioenerg Biomembr, 31*(5), 517-525.

Suehiro, T., Ohguro, T., Sumiyoshi, R., Yasuoka, N., Nakauchi, Y., Kumon, Y., Hashimoto, K. (1995). Relationship of low-density lipoprotein particle size to plasma lipoproteins, obesity, and insulin resistance in Japanese men. *Diabetes Care, 18*(3), 333-338.

Suskin, N., McKelvie, R.S., Burns, R.J., Latini, R., Pericak, D., Probstfield, J., Rouleau, J.L., Sigouin, C., Solymoss, C.B., Tsuyuki, R., White, M., Yusuf, S. (2000). Glucose and insulin abnormalities relate to functional capacity in patients with congestive heart failure. *Eur Heart J. , 21*(16), 1368-1375.

Surwit, R. S., Feinglos, M. N., Rodin, J., Sutherland, A., Petro, A. E., Opara, E. C., Kuhn, C.M., Rebuffé-Scrive, M. (1995). Differential effects of fat and sucrose on the development of obesity and diabetes in C57BL/6J and A/J mice. *Metabolism, 44*(5), 645-651.

Swan, J. W., Walton, C., Godsland, I.F., Clark, A.L., Coats, A.J., Oliver, M.F. (1994). Insulin resistance in chronic heart failure. *Eur Heart J. , 15*(11), 1528-1532.

Takahashi, N., Li, F., Hua, K., Deng, J., Wang, C. H., Bowers, R. R., Bartness, T.J.,

Kim, H.S., Harp, J.B. (2007). Increased energy expenditure, dietary fat wasting, and resistance to diet-induced obesity in mice lacking renin. *Cell Metab, 6*(6), 506-512.

Tarka, S. M., Jr., Applebaum, R. S., and Borzelleca, J. F. (1986). Evaluation of the teratogenic potential of cocoa powder and theobromine in New Zealand White rabbits. *Food Chem Toxicol, 24*(5), 363-374.

Tayek, J. A., Bistrian, B. R., Hehir, D. J., Martin, R., Moldawer, L. L., and Blackburn, G. L. (1986). Improved protein kinetics and albumin synthesis by branched chain amino acid-enriched total parenteral nutrition in cancer cachexia. A prospective randomized crossover trial. *Cancer, 58*(1), 147-157.

Thiels, C. (2008). Forced treatment of patients with anorexia. *Curr Opin Psychiatry, 21*(5), 495-498.

Thom, E. (2007). The effect of chlorogenic acid enriched coffee on glucose absorption in healthy volunteers and its effect on body mass when used long-term in overweight and obese people. *J Int Med Res, 35*(6), 900-908.

Thomas, D. R. (2007). Loss of skeletal muscle mass in aging: examining the relationship of starvation, sarcopenia and cachexia. *Clin Nutr, 26*(4), 389-399.

Tian, W. X., Li, L. C., Wu, X. D., and Chen, C. C. (2004). Weight reduction by Chinese medicinal herbs may be related to inhibition of fatty acid synthase. *Life Sci, 74*(19), 2389-2399.

Tisdale, M. J. (1997). Biology of Cachexia. *Journal of the National Cancer Institute, 89*(23), 1763-1773.

Tisdale, M. J. (2000). Metabolic abnormalities in cachexia and anorexia. *Nutrition, 16*(10), 1013-1014.

Tisdale, M. J. (2002). Cachexia in cancer patients. *Nat Rev Cancer, 2*(11), 862-871.

Tisdale, M. J. (2003). Pathogenesis of cancer cachexia. *J Support Oncol, 1*(3), 159-

168.

Tisdale, M. J. (2004). Cancer cachexia. *Langenbecks Arch Surg, 389*(4), 299-305.

Tocco-Bradley, R., Georgieff, M., Jones, C. T., Moldawer, L. L., Dinarello, C. A., Blackburn, G. L., Bistrian, B.R. (1987). Changes in energy expenditure and fat metabolism in rats infused with interleukin-1. *Eur J Clin invest, 17*(6), 504-510.

Todorov, P. T., Field, W. N., and Tisdale, M. J. (1999). Role of a proteolysis-inducing factor (PIF) in cachexia induced by a human melanoma (G361). *Br J Cancer, 80*(11), 1734-1737.

Todorov, P. T., McDevitt, T. M., Meyer, D. J., Ueyama, H., Ohkubo, I., and Tisdale, M. J. (1998). Purification and characterization of a tumour lipid-mobilizing factor. *Cancer Res, 58*(11), 2353-2358.

Tomaru, M., Takano, H., Osakabe, N., Yasuda, A., Inoue, K., Yanagisawa, R., Ohwatari, T., Uematsu, H. (2007). Dietary supplementation with cacao liquor proanthocyanidins prevents elevation of blood glucose levels in diabetic obese mice. *Nutrition, 23*(4), 351-355.

Toschi, T. G., Bordoni, A., Hrelia, S., Bendini, A., Lercker, G., and Biagi, P. L. (2000). The protective role of different green tea extracts after oxidative damage is related to their catechin composition. *J Agric Food Chem, 48*(9), 3973-3978.

Toth, M. J., Poehlman, E.T. (1994). Sympathetic nervous system activity and resting metabolic rate in vegetarians. *Metabolism. , 43*(5), 621-625.

Toth, E., Ferenc, V., Meszaros, S., Csupor, E., and Horvath, C. (2005). [Effects of body mass index on bone mineral density in men]. *Orv Hetil, 146*(28), 1489-1493.

Toth, M. J., Gottlieb, S. S., Goran, M. I., Fisher, M. L., and Poehlman, E. T. (1997). Daily energy expenditure in free-living heart failure patients. *Am J Physiol, 272*(3 Pt 1), E469-475.

Toth, M. J., and Matthews, D. E. (2006). Whole-body protein metabolism in chronic heart failure: relationship to anabolic and catabolic hormones. *JPEN J Parenter Enteral Nutr, 30*(3), 194-201.

Tracey, K. J., Wei, H., Manogue, K. R., Fong, Y., Hesse, D. G., Nguyen, H. T., Kuo, G.C., Beutler, B., Cotran, R,S., Cerami, A. (1988). Cachectin/tumour necrosis factor induces cachexia, anemia, and inflammation. *J Exp Med, 167*(3), 1211-1227.

Tsujinaka, T., Fujita, J., Ebisui, C., Yano, M., Kominami, E., Suzuki, K., Tanaka, K., Katsume, A., Ohsugi, Y., Shiozaki, H., Monden, M. (1996). Interleukin 6 receptor antibody inhibits muscle atrophy and modulates proteolytic systems in interleukin 6 transgenic mice. *J Clin invest, 97*(1), 244-249.

Uno, K., Katagiri, H., Yamada, T., Ishigaki, Y., Ogihara, T., Imai, J., Hasegawa, Y., Gao, J., Kaneko, K., Iwasaki, H., Ishihara, H., Sasano, H., Inukai, K., Mizuguchi, H., Asano, T., Shiota, M., Nakazato, M., Oka, Y. (2006). Neuronal Pathway from the Liver Modulates Energy Expenditure and Systemic Insulin Sensitivity *Science, 312*(5780), 1656-1659.

Urel, W., Hulsewe, E., Nicolaas E. P. Deutz, De Blaauw, I., Rene, R., Van Der Hulst, Maarten, W.J., Von Meyenfeldt, M.F., and Soeters, P.B. (1997). 'Liver protein and glutamine metabolism during cachexia'. *Proceedings of the Nutrition Society 56,* 801-806

Vaisman, N., Silverberg, D. S., Wexler, D., Niv, E., Blum, M., Keren, G., Soroka, N., Iaina, A. (2004). Correction of anemia in patients with congestive heart failure increases resting energy expenditure. *Clin Nutr, 23*(3), 355-361.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T., Mazur, M., and Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. In*t J Biochem Cell Biol, 39*(1), 44-84.

Van Keulen, J.V. and B.A. Young. 1977. Evaluation of acid insoluble ash as a natural marker in ruminant digestibility studies. *J. Animal. Sci. 44,* 282.

Vats, V., Yadav, S. P., and Grover, J. K. (2003). Effect of T. foenumgraecum on glycogen content of tissues and the key enzymes of carbohydrate metabolism. *J Ethnopharmacol, 85*(2-3), 237-242.

Verduyn, S. C., Ramakers, C., Snoep, G., Leunissen, J. D., Wellens, H. J., and Vos, M. A. (2001). Time course of structural adaptations in chronic AV block dogs: evidence for differential ventricular remodeling. *Am J Physiol Heart Circ Physiol, 280*(6), H2882-2890.

Vertuani, S., Angusti, A., and Manfredini, S. (2004). The antioxidants and pro-antioxidants network: an overview. *Curr Pharm Des, 10*(14), 1677-1694.

Vescovo, G., Volterrani, M., Zennaro, R., Sandri, M., Ceconi, C., Lorusso, R., Ferrari, R., Ambrosio, G.B., Dalla Libera, L. (2000). Apoptosis in the skeletal muscle of patients with heart failure: investigation of clinical and biochemical changes. *Heart, 84*(4), 431-437.

Vidal-Puig, A. J. (2000). Uncoupling expectations. *Nat Genet, 26*(4), 387-388.

Vignes, M., Maurice, T., Lante, F., Nedjar, M., Thethi, K., Guiramand, J., Récasens, M. (2006). Anxiolytic properties of green tea polyphenol (-)-epigallocatechin gallate (EGCG). *Brain Res, 1110*(1), 102-115.

Volmert, R. F., and Firman, J. D. (1991). Water and NaCl intake of chicks as mediated by Angiotensin II, renin, or salt deficiency. *Physiol Behav, 50*(5), 921-927.

von Haehling, S., Doehner, W., and Anker, S. D. (2007). Nutrition, metabolism, and the complex pathophysiology of cachexia in chronic heart failure. *Cardiovasc Res, 73*(2), 298-309.

Watras, A. C., Buchholz, A. C., Close, R. N., Zhang, Z., and Schoeller, D. A. (2007).

The role of conjugated linoleic acid in reducing body fat and preventing holiday weight gain. In*t J Obes (Lond), 31*(3), 481-487.

Weisburger, J. H. (2001). Chemopreventive effects of cocoa polyphenols on chronic diseases. *Exp Biol Med (Maywood), 226*(10), 891-897.

Weisinger, R. S., Begg, D. P., Chen, N., Jois, M., Mathai, M. L., and Sinclair, A. J. (2007). The problem of obesity: is there a role for antagonists of the renin-Angiotensin system? *Asia Pac J Clin Nutr, 16 Suppl 1*, 359-367.

Wetmore, C. M., Ichikawa, L., LaCroix, A. Z., Ott, S. M., and Scholes, D. (2008). Association between caffeine intake and bone mass among young women: potential effect modification by depot medroxyprogesterone acetate use. *Osteoporos Int, 19*(4), 519-527.

Whitehouse, A. S., and Tisdale, M. J. (2003). Increased expression of the ubiquitin-proteasome pathway in murine myotubes by proteolysis-inducing factor (PIF) is associated with activation of the transcription factor NF-kappaB. *Br J Cancer, 89*(6), 1116-1122.

Wigmore, S. J., Todorov, P. T., Barber, M. D., Ross, J. A., Tisdale, M. J., and Fearon, K. C. (2000). Characteristics of patients with pancreatic cancer expressing a novel cancer cachectic factor. *Br J Surg, 87*(1), 53-58.

Wilcox, C. S. (2002). Reactive oxygen species: roles in blood pressure and kidney function. *Curr Hypertens Rep, 4*(2), 160-166.

William, Sis. M.W., and HESS WC. (1952). ‘A comparison of total serum protein and albumin values as determined by the micro-Kjeldahl, Biuret, and Folin methods’. *Bull Georgetown Univ Med Cent. 6(2),* 34-5.

Williams, C. J., Fargnoli, J.L., Hwang, J.J., van Dam, R.M., Blackburn, G.L., Hu, F.B., Mantzoros, C.S. (2008). Coffee consumption is associated with higher plasma

adiponectin concentrations in women with or without type 2 diabetes: a prospective cohort study. *Diabetes Care, 31*(3), 504-507. .

Witte, K. K., & Clark, A. L. (2006). Micronutrients and their supplementation in chronic cardiac failure. An update beyond theoretical perspectives. *Heart Fail Rev. 11(1)*, 65-74.

Wittels, B., and Spann, J. F., Jr. (1968). Defective lipid metabolism in the failing heart. *J Clin Invest, 47*(8), 1787-1794.

Wober, C., Holzhammer, J., Zeitlhofer, J., Wessely, P., and Wober-Bingol, C. (2006). Trigger factors of migraine and tension-type headache: experience and knowledge of the patients. *J Headache Pain, 7*(4), 188-195.

Wolfram, S., Raederstorff, D., Wang, Y., Teixeira, S. R., Elste, V., and Weber, P. (2005). TEAVIGO (epigallocatechin gallate) supplementation prevents obesity in rodents by reducing adipose tissue mass. *Ann Nutr Metab, 49*(1), 54-63.

Wolk, R., Johnson, B. D., and Somers, V. K. (2003). Leptin and the ventilatory response to exercise in heart failure. *J Am Coll Cardiol, 42*(9), 1644-1649.

Wolk, R., Shamsuzzaman, A. S., and Somers, V. K. (2003). Obesity, sleep apnea, and hypertension. *Hypertension, 42*(6), 1067-1074.

Wright, C. E., Strike, P. C., Brydon, L., and Steptoe, A. (2005). Acute inflammation and negative mood: mediation by cytokine activation. *Brain Behav immun, 19*(4), 345-350.

Wu, G. H., Liu, Z. H., Wu, Z. H., and Wu, Z. G. (2006). Perioperative artificial nutrition in malnourished gastrointestinal cancer patients. *World J Gastroenterol, 12*(15), 2441-2444.

Xu, X. B., Pang, J. J., Cao, J. M., Ni, C., Xu, R. K., Peng, X. Z., Yu, X.X., Guo, S., Chen, M.C., Chen, C. (2005). GH-releasing peptides improve cardiac dysfunction and

cachexia and suppress stress-related hormones and cardiomyocyte apoptosis in rats with heart failure. *Am J Physiol Heart Circ Physiol, 289*(4), H1643-1651.

Yamamoto, E., Lai, Z. F., Yamashita, T., Tanaka, T., Kataoka, K., Tokutomi, Y., Ito, T., Ogawa, H., Kim-Mitsuyama, S. (2006). Enhancement of cardiac oxidative stress by tachycardia and its critical role in cardiac hypertrophy and fibrosis. *J Hypertens, 24*(10), 2057-2069.

Yamamoto, K., Burnett, J. C., Jr., Meyer, L. M., Sinclair, L., Stevens, T. L., and Redfield, M. M. (1996). Ventricular remodeling during development and recovery from modified tachycardia-induced cardiomyopathy model. *Am J Physiol, 271*(6 Pt 2), R1529-1534.

Yang, M., Wang, C., and Chen, H. (2001). Green, oolong and black tea extracts modulate lipid metabolism in hyperlipidemia rats fed high-sucrose diet. *J Nutr Biochem, 12*(1), 14-20.

Yang, T. T., and Koo, M. W. (2000). Chinese green tea lowers cholesterol level through an increase in fecal lipid excretion. *Life Sci, 66*(5), 411-423.

Yu, X. X., Barger, J. L., Boyer, B. B., Brand, M. D., Pan, G., and Adams, S. H. (2000). Impact of endotoxin on UCP homolog mRNA abundance, thermoregulation, and mitochondrial proton leak kinetics. *Am J Physiol Endocrinol Metab, 279*(2), E433-446.

Yuan, M., Konstantopoulos, N., Lee, J., Hansen, L., Li, Z. W., Karin, M., Shoelson, S.E. (2001). Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of Ikkbeta. *Science, 293*(5535), 1673-1677.

Zaloga, G. P. (2006). Parenteral nutrition in adult inpatients with functioning gastrointestinal tracts: assessment of outcomes. *Lancet, 367*(9516), 1101-1111.

Zeman, R. J., Peng, H., Danon, M. J., and Etlinger, J. D. (2000). Clenbuterol reduces

degeneration of exercised or aged dystrophic (mdx) muscle. *Muscle Nerve, 23*(4), 521-528.

Zheng, G., Sayama, K., Okubo, T., Juneja, L. R., and Oguni, I. (2004). Anti-obesity effects of three major components of green tea, catechins, caffeine and theanine, in mice. In *Vivo, 18*(1), 55-62.

Zia, T., Hasnain, S. N., and Hasan, S. K. (2001). Evaluation of the oral hypoglycaemic effect of Trigonella foenum-graecum L. (methi) in normal mice. *J Ethnopharmacol, 75*(2-3), 191-195.

Zinna, E. M., and Yarasheski, K. E. (2003). Exercise treatment to counteract protein wasting of chronic diseases. *Curr Opin Clin Nutr Metab Care, 6*(1), 87-93.