EFFECTS OF CARALLUMA FIMBRIATA EXTRACT ON CARDIOVASCULAR AND METABOLIC DISORDERS

A thesis submitted by

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ABSTRACT

Central obesity, insulin resistance, atherogenic dyslipidemia and elevated blood pressure are the major components of metabolic syndrome. This complex disorder is considered to be a clinical challenge and an urgent public health issue. With the growing prevalence of obesity worldwide, effective strategies are needed to intervene in the development and progression of metabolic syndrome. Despite the short-term benefits of pharmaceutical treatment of obesity, current drug therapy is associated with adverse side effects, thus the use of complementary and alternative therapies has become increasingly popular among the general population as an alternative method for weight loss. Botanical extracts in combination with lifestyle modification may be effective agents for attenuating the development of metabolic syndrome as they often comprise of a vast range of bioactive compounds that have been associated with significant positive health outcomes with minimal side effects. However, the efficacy of many of these extracts and their chemical constituents have yet to be fully explored. The research presented in this thesis examines the effectiveness of two commonly used anti-obesity botanical extracts, namely *Caralluma fimbriata* and *Citrus sinensis* (Moro variety). The primary aim of this PhD project was to investigate the efficacy of *C. fimbriata* extract on the risk factors of metabolic syndrome in overweight and obese conditions.

The first study was a 12-week pilot study that sought to determine whether *C. fimbriata* extract (1 g/day), in addition to a hypocaloric diet and regular physical activity, can attenuate metabolic disturbances in overweight and obese individuals compared to placebo (n = 33; 29-59 years old; 26 females, 7 males; BMI: 32.15 kg/m^2). The main outcome was a significant reduction in waist circumference in the experimental group (mean change: -3.847; 95 % CI; -7.466 – 0.228). This study identified that supplementation with *C. fimbriata* extract may potentially play a role in curbing central obesity, the key component of metabolic syndrome.

The second study then aimed to determine whether *C. fimbriata* extract attenuates the metabolic changes developed in an obesity-inducing rat model (n = 40; 4 weeks old; body weight: 229.7 g). This study examined metabolic effects of chronic administration of *C. fimbriata* extract (100 mg/kg BW) in male wistar rats with diet induced obesity. This study revealed that treatment with *C. fimbriata* extract for eight weeks in lean & high fat fed rats does not have significant effects on feed intake, obesity, glucose tolerance, blood pressure and lipid profile ($p > 0.05$).

The final study aimed to explore the effect of *C. fimbriata* extract (1g/day) alone and in combination with *C. sinensis* extract (500 mg/day) on metabolic and cardiovascular risk factors in a randomised controlled clinical trial (n = 59; 46.6 years old; 19 males, 40 females; BMI: 34.3 kg/m^2). The main finding was a significant time effect observed in all groups for body composition, food intake and lipid profile ($p < 0.05$), which might be attributed to the dietary advice provided. However, there was no significant effect observed for all data on body composition, dietary intake, cardiovascular parameters, appetite sensations and lipid profile ($p > 0.05$).

This research has made a significant contribution to the literature, providing evidence that *C. fimbriata* extract and/or *C. sinensis* extract may not be effective in eliciting beneficial effects in metabolic and cardiovascular conditions. Furthermore, the dietary modifications to participants' food intake were clinically meaningful, providing significant improvements to body composition parameters. In conclusion, the results presented within the current thesis do not support that botanical extracts, including *C. fimbriata* extract and *C. sinensis* extract play a significant role in the treatment of metabolic abnormalities, due to the inconsistencies of data identified within this thesis and previous work.

CANDIDATE DECLARATION

"I, Katie Astell, declare that the PhD thesis entitled, Effects of *Caralluma fimbriata* extract on cardiovascular and metabolic disorders is no more than 100,000 words in length including quotes and exclusive of tables, figures, appendices, bibliography, references and footnotes. This thesis contains no material that has been submitted previously, in whole or in part, for the award of any other academic degree or diploma. Except where otherwise indicated, this thesis is my own work".

Signature: Date:

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ABBRIVIATIONS

4-AAP	4-aminoantipyrine
ACE	Angiotensin converting enzyme
AEC	Animal Ethics Committee
AgRP	Agouti-related protein
AIP	Atherogenic index of plasma
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
ANZCTR	Australian New Zealand Clinical Trials Registry
ASF	Abdominal subcutaneous fat
ATP	Adenosine triphosphate
AVF	Abdominal visceral fat
BDNF	Brain-derived neurotropic factor
BioLED	Biomedical and Lifestyle Diseases Unit
BMI	Body mass index
BP	Blood pressure
CART	Cocaine and amphetamine-regulated transcript
CDK	Cyclin-dependent kinase
CETP	Cholesteryl ester transfer protein
CFE	Caralluma fimbriata extract
CHD	Coronary heart disease
CRP	C-reactive protein
CT-scan	Computed tomography scan
CVD	Cardiovascular disease
DALYs	Disability-adjusted life years
DAP	Dihydroxyacetone phosphate

3, 5 DHBS	3,5-dichloro-2-hydroxybenzene sulfonate
DQESV2	Dietary Questionnaire for Epidemiological Studies Version 2
EDTA	Ethylenediaminetetraacetic acid
EGIR	European Group for the Study of Insulin Resistance
ELISA	Enzyme linked immunosorbent assay
FFA	Free fatty acids
FFQ	Food Frequency Questionnaire
GGT	γ-glutamyltransferase
GLP	Good laboratory Practice
GPO	Glycerolphosphate oxidase
HBA	Hydroxbenzoic acid
HCA	Hydroxycitric acid
HDAOS	N-(2-hydroxy-3-sulfoprophyl)-3,5-dimethoxyaniline
HDL	High density lipoprotein
H & E	Haematoxylin and Eosin
HK	Hexokinase II
HOMA	Homeostatic model assessment
HREC	Human Research Ethics Committee
HRP	horseradish peroxidase
ICAM-1	Intercellular adhesion molecule-1
IDF	International Diabetes Federation
IL-6	Interleukin 6
IPAQ	International Physical Activity Questionnaire
IPGTT	Intraperitoneal glucose tolerance test
LDL	Low density lipoprotein
NAFLD	Non-alcoholic fatty liver disease

NBF	Neutral-buffered formalin
NO	Nitrogen oxide
NHMRC	National Health and Medical Research Council
Ox-LDL	Oxidized LDL
PAI-1	plasminogen activator inhibitor type 1
PCR	Polymerase chain reaction
PDH	Pyruvate dehydrogenase
PFK	Phosphofructokinase
PKCθ	Protein kinase Cθ
POD	Peroxidase
POMC	Pro-opiomelanocortin
PPARs	Peroxisome proliferator-activated receptors
RAAS	Renin-angiotensin-aldosterone system
RCTs	Randomised controlled trials
RIA	Radioimmunoassay
ROS	Reactive oxygen species
SQ	Satiety quotient
STD CHOW	Standard chow
TAF	Total abdominal fat
TMB	Tetramethylbenzidine
TNF-α	Tumour necrosis factor alpha
VAS	Visual analogue scale
VCAM-1	Vascular cell adhesion molecule-1
VLDL	Very low density lipoprotein
WAT	White adipose tissue
WHO	World Health Organisation
WHR	Waist to hip ratio

PUBLICATIONS AND PRESENTATIONS DURING CANDIDATURE

Peer reviewed publications:

ASTELL, K. J., MATHAI, M. L., MCAINCH, A. J., STATHIS, C. G. & SU, X. Q. 2013. A pilot study investigating the effect of *Caralluma fimbriata* extract on the risk factors of metabolic syndrome in overweight and obese subjects: a randomised controlled clinical trial. *Complement Ther Med*, 21**,** 180-9.

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ASTELL, K. J., MATHAI, M. L. & SU, X. Q. 2013. Plant extracts with appetite suppressing properties for body weight control: a systematic review of double blind randomized controlled clinical trials. *Complement Ther Med,* 21**,** 407-16.

LOFTUS, H.L, **ASTELL, K.J,** MATHAI, M.L & SU, X.Q. Coleus forskohlii extract supplementation in conjunction with a hypocaloric diet reduces the risk factors of metabolic syndrome in overweight and obese subjects: a randomised controlled trial. *Nutrients,* 7, 9508-22.

Oral presentations:

The effect of *Caralluma fimbriata* extract in combination with lifestyle intervention on the risk factors of metabolic syndrome *(Presented at the School of Exercise & Nutrition Sciences 8th Annual Research Degree Symposium, Deakin University, Melbourne, October 2011).*

A pilot study investigating the effect of *Caralluma fimbriata* extract on the risk factors of metabolic syndrome in overweight and obese subjects: a randomised controlled clinical trial *(Presented at Biomedical and Lifestyle Diseases Unit (BioLED) Scientific Meeting, Victoria University, Western Centre for Health Research & Education, Sunshine Hospital, Melbourne, April 2013).*

The effect of *Caralluma fimbriata* extract on an obesity-inducing rat model (*Presented at Victoria University, College of Health and Biomedicine Postgraduate Student Research Conference, October 2014).*

Poster Presentations:

The effect of *Caralluma fimbriata* extract in combination with lifestyle intervention on the risk factors of metabolic syndrome *(Presented at the School of Biomedical and Health Sciences Conference, Victoria University, Melbourne, July 2011).*

The effect of *Caralluma fimbriata* extract in combination with lifestyle intervention on the risk factors of metabolic syndrome (*Presented at the Joint Annual Scientific Meeting of the Nutrition Society of New Zealand & the Nutrition Society of Australia, New Zealand, November 2011).*

The effect of *Caralluma fimbriata* extract on metabolic parameters in high fat fed Wistar rats (*Presented at The Annual Scientific Meeting of the Nutrition Society of Australia, Hobart, November 2014).*

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CHAPTER 1: INTRODUCTION

Metabolic syndrome is defined as the coexisting occurrence of a clustering of metabolic conditions, including central obesity, insulin resistance, dyslipidemia, and hypertension in an individual which leads to an increased risk in the development of cardiovascular disease (CVD), type 2 diabetes and stroke (Beltran-Sanchez et al., 2013). This clustering of metabolic disorders is of substantial concern, as the incidences of both CVD and type 2 diabetes have reached epidemic proportions worldwide. Metabolic syndrome is quite a complex and progressive condition that has the potential to develop over several years with the characteristics and extent of the disorder varying between individuals (Graf et al., 2010).

With the rapid increase in the incidence and prevalence of metabolic syndrome in several parts of the world coupled with the increase in life expectancy and associated changes in demographics, it continues to challenge the resourcefulness of scientists and clinicians in refining current therapies and the development of new strategies to counteract this prevalent condition. Botanical extracts may serve as effective agents for the management and treatment of metabolic syndrome because they contain mixtures of interacting compounds reputed to possess important combination therapies such as anti-obesity, anti-diabetic, anti-inflammatory, anti-hypertensive and lipid lowering properties. These multicomponent botanical extracts may in turn act simultaneously to affect multiple pharmacological targets and therefore inaugurate clinical efficacy beyond the extent of single compound based pharmaceutical drugs. Therefore it is possible that the complexity of metabolic syndrome may be addressed via a prophylactic strategy comprising of bioactive compounds. However, many botanical extracts have not received equitable scientific/medical scrutiny, in addition to only a small number of purportedly bioactive constituents found in these botanical extracts being wholly or partially characterized (Astell et al., 2013b).

Although, it should be remembered that there is a great proportion of the world's flora that is yet to be scientifically investigated and therefore it is highly plausible that this immerse botanical resource may produce new drug leads that can add to the repertoire of obesity and diabetes research. Future research should focus on developing innovative scientific methods for the discovery, characterisation, validation, and standardisation of these multicomponent botanical extracts as it is vital for their acceptance into mainstream medicine. The scope for the discovery and development of new target therapies for the prevention and management of metabolic syndrome from nature's pharmacy is vast, which merits corresponding consideration.

This thesis investigated the efficacy of two botanical extracts, known as *Caralluma fimbriata* extract and *Citrus sinensis* extract (Moro variety) as a therapeutic target for the management and treatment of metabolic syndrome. The clinical efficacy of these two botanical extracts in the treatment of associated metabolic disturbances is yet to be validated. It is unknown whether *C. fimbriata* extract is capable of alleviating all components of metabolic syndrome in overweight and obese humans. Hence, study one investigated the effectiveness of *C. fimbriata* supplementation on the risk factors of metabolic syndrome including central obesity, elevated blood glucose levels, high blood pressure and dyslipidemia in overweight and obese adults.

There is sparse research on the metabolic effects of *C. fimbriata* extract in rodents. To better understand the clinical utility of *C. fimbriata* in the treatment of metabolic syndrome, the underlying pathologies of the syndrome following *C. fimbriata* administration in an obese state in rodents as a translatable model of the etiopathology of the human condition is required. Thus, as it was apparent that further research was needed in determining the effectiveness of *C. fimbriata* administration on the functional and structural abnormalities

associated with metabolic syndrome, study two investigated the efficacy of *C. fimbriata* administration in an animal model of diet induced obesity on organ weight, liver histology, body composition, appetite, cholesterol and triglyceride levels, diabetes risk and hypertension.

A previous animal model of obesity following the ingestion of *C. fimbriata* extract identified potential anti-atherosclerotic properties in high fat cafeteria fed rodents (Kamalakkannan et al., 2010). However, it is unclear as to whether supplementation with *C. fimbriata* in humans would play a role in the treatment of inflammatory and atherosclerotic disease in humans. It was therefore of significance to investigate the effect of *C. fimbriata* extract on vascular parameters associated with metabolic syndrome, inflammation and atherosclerosis in humans. It was also of interest to validate the findings observed in our previous work to better clarify the anti-obesity and appetite supressing effects of *C. fimbriata* extract.

Furthermore, potential anti-inflammatory and antioxidant properties have been identified in anthocyanin plant-derived extracts in humans (Dallas et al., 2014, Buscemi et al., 2012) and in vitro studies (Cardile et al., 2010). Importantly, there is an established link between chronic inflammation and metabolic disorders (Monteiro and Azevedo, 2010), therefore it was of significance to investigate the effect of the anthocyanin rich red orange extract (*C. sinensis*) on inflammatory biomarkers of metabolic syndrome. In addition, the anti-obesity effects of the Moro orange variety have been elucidated in animal models of diet induced obesity, however there is limited clinical evidence on the efficacy of *C. sinensis* extract on the risk factors of metabolic syndrome in overweight and obese humans. Hence, study three explored the clinical effectiveness of *C. fimbriata* and *C. sinensis* supplementation on obesity, metabolic syndrome and atherosclerotic indices in overweight and obese adults.

CHAPTER 2: REVIEW OF LITERATURE

SECTION I: AN INTRODUCTION TO METABOLIC SYNDROME

2.1.1 Metabolic syndrome definition

Metabolic syndrome is a global health problem defined by a constellation of clinical criteria used to identify patients at an increased risk of atherosclerotic CVD, type 2 diabetes and all-cause mortality (Beltran-Sanchez et al., 2013). Over the next 5-10 years, metabolic syndrome confers a 5-fold increased risk in the development of type 2 diabetes and a 2-fold increased risk of developing cardiovascular disease (Alberti et al., 2009). In addition, there is a 2-4 fold increased risk of stroke, 3-4 fold increased risk of myocardial infarction and 2-fold increased risk of dying from such an event in patients with metabolic syndrome (Alberti et al., 2006).

Metabolic syndrome originated from the observation of several metabolic risk factors that are interconnected physiologically, biochemically, clinically and metabolically in patients at high risk of cardiovascular disease, which comprise of: central obesity, atherogenic dyslipidemia, elevated plasma glucose levels and elevated blood pressure (The National Cholesterol Education Program, 2001). Metabolic syndrome is a complex disorder that is considered to be a clinical challenge and an imperative public health issue. Metabolic syndrome is described as a state of low grade inflammation, and is a consequence of a series of complex interactions which consist of unhealthy lifestyle habits, genetics and environmental influences and the dysregulation of hormones. There are many factors which constitute this syndrome which include insulin resistance, visceral adiposity, elevated blood pressure, dyslipidemia, endothelial dysfunction, genetic susceptibility, hypercoagulable state, prothrombotic state and chronic stress (Kaur, 2014) (Figure 2.1; Taken from Kaur 2014).

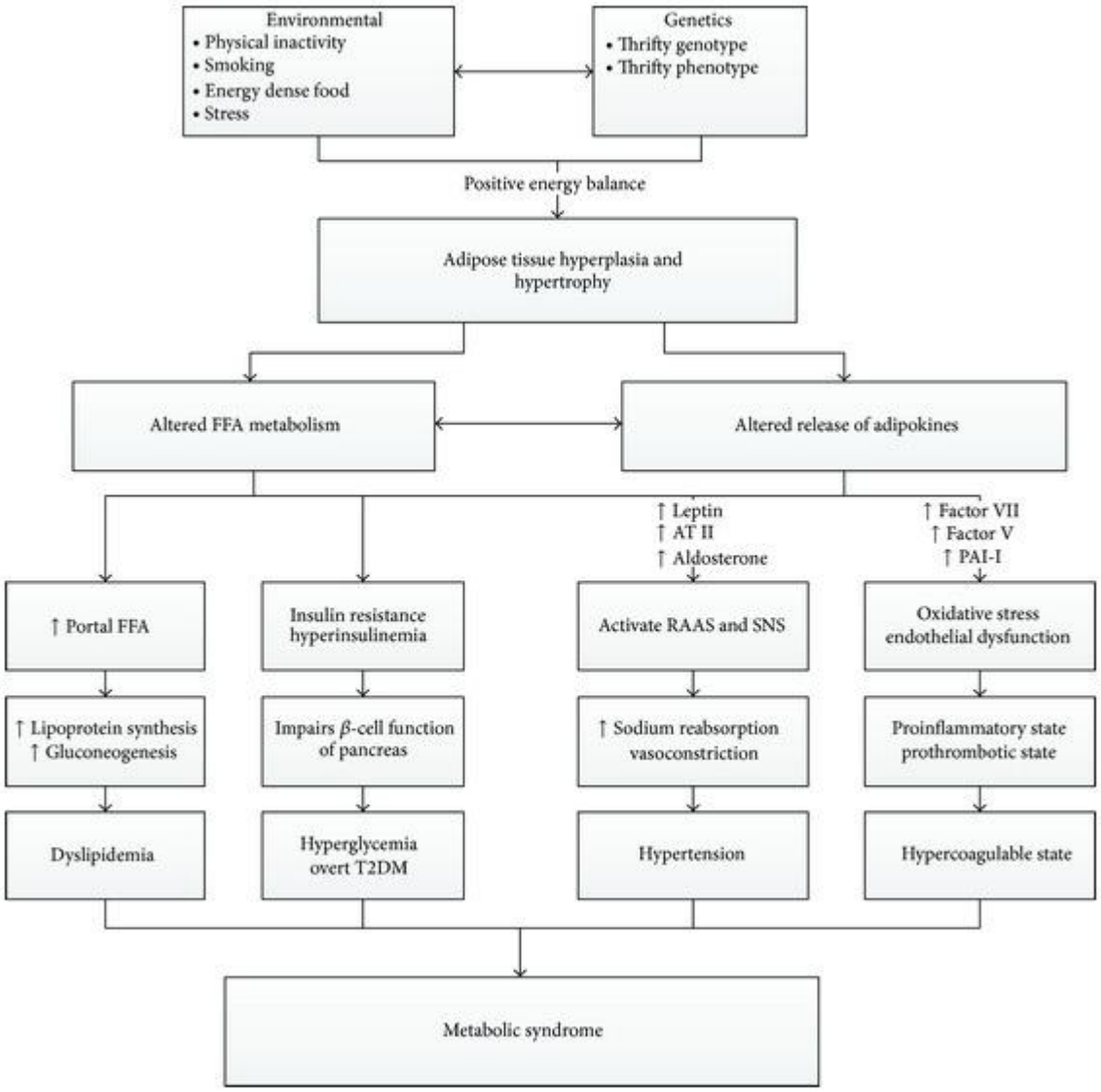


Figure 2.1: Schematic presentation of metabolic syndrome (Kaur, 2014)

Given the complexities of the factors leading to the development of metabolic syndrome, the definition of metabolic syndrome has sparked much controversy, resulting in several current definitions (Cameron et al., 2007). The complex nature of the metabolic syndrome presents significant methodological challenges over which single factor amongst the several inter-related abnormalities is present in all the conditions of metabolic syndrome and provides the link that unifies them as the central abnormality (Anderson et al., 2001).

The first attempt of a global definition of metabolic syndrome was proposed by the World Health Organization (WHO) in 1999 (World Health Organization, 1999). The biological and physiological description of insulin resistance (measured by the euglycaemic clamp) was pivotal to the WHO definition. Initially this definition was identified as a working model, where the authors acknowledged that the definition will need to be improved following the publication of new data (Balkau and Charles, 1999). Several limitations of the WHO definition were identified by critics. The major limitations related to the use of euglycaemic clamp for the measurement of insulin sensitivity, therefore reducing the efficacy in a clinical setting or for epidemiological studies. Consequently, the WHO definition has been deemed as too complex to use in many settings. Therefore in large scale studies, the euglycaemic clamp method of measuring insulin resistance has been substituted with the calculation of insulin sensitivity via the homeostatic model assessment (HOMA). The European Group for the Study of Insulin Resistance (EGIR) established a modified version of the WHO definition which would be accessible and easy to use as fasting insulin was implemented instead of the euglyceamic clamp to measure insulin resistance (Einhorn et al., 2003).

Two years later, the USA National Cholesterol Education Program created the ATPIII definition (The National Cholesterol Education Program, 2001). The definition was intended to have clinical utility; it did not incorporate a specific measure of insulin sensitivity and implemented a less glucose centric approach by treating all metabolic components with equal significance. The ATPIII definition is commonly used as it is simplistic and easy to use in the research and clinical setting.

Considerable confusion has developed as a result of these definitions of metabolic syndrome. Clinicians have highlighted the various limitations of the current definitions and have described the definitions as being not ‘user friendly’. Therefore the International Diabetes

Federation (IDF) developed a more practical definition which would be a clinically useful diagnosis tool and applicable globally for the assessment of individuals at high risk of type 2 diabetes and CVD (Alberti et al., 2006). The IDF group recognized that central obesity was a significant determinant of metabolic syndrome and that waist circumference has a strong link with other components of metabolic syndrome. Therefore central obesity was identified as an essential component of the new definition. Table 2.1 shows the current definitions of metabolic syndrome (Cameron et al., 2007).

Table 2.1: Current definitions of metabolic syndrome

WHO 1999	EGIR 1999	ATPIII 2001	IDF 2005
Diabetes or impaired glucose tolerance or insulin resistance	Insulin resistance or hyperinsulinemia (only non-diabetes subjects)		Central obesity Ethnicity specific waist circumference cut-off points
Plus two or more of the following:	*Plus two or more of the following:*	*Three or more of the following:*	*Plus any two of the following:*
Obesity: BMI >30 kg/m^2 or waist: hip ratio >0.9 (male), >0.85 (female)	Central obesity: waist circumference >94 cm (m), >80 cm (f)	Central obesity: waist circumference >102 cm (m), >88 cm (f)	
Dyslipidemia: Triglycerides >1.7 mmol/L or HDL-C <0.9 mmol/L (m), <1.0 mmol/L (f)	Dyslipidemia: triglycerides >2.0 mmol/L or HDL-C <1.0 mmol/L	Hypertriglyceridemia: Triglycerides >1.7 mmol/L Low HDL-C: <1.03 mmol/L (m) 1.29 mmol/L (f)	Raised triglycerides >1.7 mmol/L or specific treatment for this abnormality Reduced HDL-C <1.03 mmol/L (m) 1.29 mmol/L (f) or specific treatment for this abnormality
Hypertension: Blood pressure >140/90 mmHg or medication	Hypertension: Blood pressure >140/90mmHg or medication	Hypertension: Blood pressure >130/85 mmHg or medication	Hypertension: >130/85 mmHg or medication
Microalbuminuria: Albumin excretion >2.5 mg/mmol/L (m) and >3.5 mg/mmol/L (f)	Fasting plasma glucose >6.1 mmol/L	Fasting plasma glucose >6.1 mmol/L	Fasting plasma glucose >5.6 mmol/L or previously diagnosed type 2 diabetes

2.1.2 The concept of metabolic syndrome

A universal definition of metabolic syndrome is yet to be established. The concept of metabolic syndrome was proposed to emphasize the simultaneous presence of risk factors for type 2 diabetes and CVD (Ferrannini et al., 1987). The origin of metabolic syndrome was initially identified in 1920, when Kylin a Swedish physician described a syndrome involving the clustering of hypertension, hyperglycaemia and hyperuricaemia or gout (Kylin, 1923). In 1947, Vague reported that abdominal obesity (android obesity) was linked with metabolic abnormalities often seen in type 2 diabetes and CVD (Vague, 1947). In 1965, Avogaro and Crepaldi described a syndrome characterised by obesity, diabetes, hyperlipidemia and hypertension (Avogaro, 1965).

The term "Metabolic syndrome" was first used in the 1970s by German researchers who also explored the link between metabolic syndrome and atherosclerosis. In the late 1980s, insulin resistance was suggested to be the underlying cause of metabolic syndrome (Reaven, 1988, Ferrannini et al., 1987). Consequently, Ferrannini et al., (1987) preferred the term "Insulin resistance syndrome" while Reaven (1988) used the term "Syndrome X" (Isomaa et al., 2001, Alexander et al., 2003, Grundy et al., 2004). In 1989, Kaplan renamed the syndrome "The Deadly Quartet" (Kaplan, 1989) and by 1992, the syndrome was again renamed "The Insulin Resistance Syndrome" (Haffner et al., 1992). Currently the term "Metabolic Syndrome" now remains the universal description of this cluster of metabolic abnormalities.

2.1.3 Prevalence of metabolic syndrome in Australia

Cameron et al., (2007) compared the four definitions of metabolic syndrome to determine which one is the best in identifying those at high CVD risk with insulin resistance. The results showed that using any of the four definitions, the prevalence of metabolic syndrome is high in Australian adults aged >25 years in 1999-2000 (n = 11,247; 5049 men, 6198 women). Approximately one in three adults were classified as having metabolic syndrome according to the IDF definition, and one in five by the ATPIII and the WHO definitions. Slightly less were defined as having metabolic syndrome by the EGIR definition (Cameron et al., 2007).

The prevalence of metabolic syndrome using the IDF definition is significantly higher than other definitions, reflecting differences in the elements incorporated i.e. core components and the design of each definition. A core feature of the IDF definition is abdominal obesity with different cut-off points for waist circumference which is dependent on ethnicity (Cameron et al., 2007). In contrast, impaired glucose regulation and insulin resistance are core components of the WHO and EGIR definitions. The IDF consensus group placed emphasis on developing criteria for abdominal obesity which would be applicable for a wide variety of populations. In different ethnic groups, central obesity is not sufficiently detected using BMI. Clinical trials in Asia have found that type 2 diabetes and CVD risk for those with metabolic syndrome is evident at a much lower level of adiposity than Europids (Caucasian European origin) (Lackland et al., 1992). Furthermore, validation for ethnic-specific cut-off points was reported in Japan which demonstrated that waist circumference cut-off points of 90 cm in men and 85 cm in women were more pertinent to this community than the ATPIII criteria (Matsuzawa, 1997). Therefore the IDF criterion is more applicable with the inclusion of cut-off points of waist circumference to different ethnic groups.

Findings of the Cameron et al., (2007) study are consistent with previous trials. Meigs et al., (2003) demonstrated that participants with metabolic syndrome defined by any definition were more insulin resistant and at an increased risk of coronary heart disease (CHD) than individuals without metabolic syndrome (11.8 vs. 6.4 % *p* 0.0001) (Meigs et al., 2003). The high prevalence of metabolic syndrome emphasizes the need for preventative strategies for co-morbidities associated with metabolic syndrome including diabetes and CVD.

Amongst people with metabolic syndrome, more than 2/3 of high risk CVD (>15 % CVD risk) could be eradicated if metabolic syndrome did not occur. For the total survey population (aged 35-74) and those free of CVD, 9-23 % of high CVD risk could have been avoided if metabolic syndrome did not develop (Cameron et al., 2007). The highest percentage of metabolic syndrome was reported with the IDF definition, signifying it may offer the greatest use in helping to prevent CVD. Altering the high risk population to a low risk population therefore may provide considerable health gains and reduce the burden of disease (Cameron et al., 2007).

2.1.4 Global prevalence of metabolic syndrome

It is difficult to compare the prevalence of metabolic syndrome in different populations even though a global definition of metabolic syndrome is in reach of agreement (Cameron et al., 2004). Several studies compare the prevalence of metabolic syndrome using different criteria. Therefore a standardised international definition needs to be reinforced. Figure 2.2 shows the prevalence of metabolic syndrome from several countries (Cameron et al., 2004). The studies differ in the design, sample selection, year the study was undertaken, the specific definition of metabolic syndrome used, gender and age of the targeted population. These studies used

the National Cholesterol Education Panel ATPIII criteria which may not be appropriate for the Asian population.

It has been shown that despite the controversy concerning the use of the metabolic syndrome definition and criteria, evidence suggests that certain inferences may be made. For instance, there is a broad variation in the prevalence of metabolic syndrome in both sexes even when participants are in the same age group. Furthermore, for individuals aged 20-25 years or older, the metabolic syndrome prevalence varies in urban populations from 7 % in French and 43 % in Iranian women and 8 % in Indians and 24 % in American men. Ethnic origin also affects the prevalence of metabolic syndrome. For example there is a higher prevalence of metabolic syndrome in Mexican Americans in comparison to Non-Hispanic white people in the USA (Ford et al., 2002). There is also a lower prevalence of metabolic syndrome in African American men compared with Mexican American men and Non-Hispanic white men (Ford et al., 2002).

A consistent finding established is that the metabolic syndrome prevalence is highly age-dependent. This pattern is established in Iran with the metabolic syndrome prevalence less than 10 % in women and men aged between 20-29 years, while the 60-69 years of age group has a prevalence of 67 and 38 % respectively (Azizi et al., 2003). Also in the French population, the prevalence of metabolic syndrome is less than 5.6 % in the 30-39 years of age group, rising to 17.5 % in the 60-64 year old group (Azizi et al., 2003). In addition, according to the National Health and Nutrition Examination Survey, the metabolic syndrome prevalence in the USA (Hispanic/Caucasian/African American) in 20-29 years was 7 % rising to 44 % in 60-69 year age bracket (Ford et al., 2002).

Prevalence of metabolic syndrome

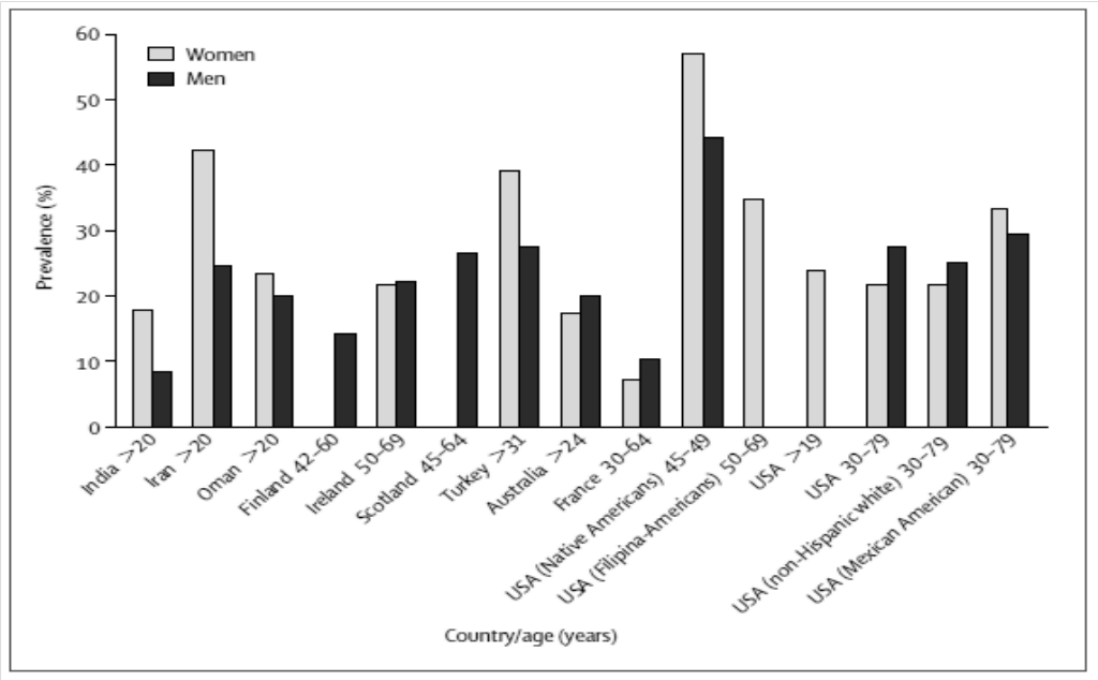


Figure 2.2: Prevalence of metabolic syndrome from ATP111 definition (Cameron et al., 2004).

SECTION II: RISK FACTORS AND MAJOR MANIFESTATIONS OF METABOLIC SYNDROME

2.2.1 Obesity

2.2.1.1 Definition and classification of obesity

Obesity is defined as a condition of excessive or abnormal fat accumulation in the adipose tissue (World Health Organization, 2000c). Excess body fat is the result of excess dietary energy intake over energy expenditure, which leads to excess kilojoules being stored as fat, ultimately resulting in overweight or obesity (World Health Organization, 2000c).

The primary classification of overweight and obesity is based on the BMI measurement, which is used to estimate relative risk for disease compared to normal weight and is

calculated with the following formula: BMI = weight (kg) / height $(m)^2$ (World Health Organization, 2000c). The classification of overweight and obesity according to BMI is presented in Table 2.2.

Table 2.2: Classification of Overweight and Obesity by BMI, Waist Circumference and Associated Disease Risks

			Disease Risk Relative to Normal BMI and Waist Circumference*	
	BMI	Obesity class	Men <102 cm (<40 in)	> 102 cm (> 40 in) kg/m^2
			Women <88 cm (<35 in)	> 88 cm (> 35 in)
Underweight	< 18.5		_	_
Normal†	18.5–24.9		_	_
Overweight	25.0–29.9		Increase	High
Obesity	30.0–34.9	I	High	Very high
	35.0–39.9	II	Very high	Very high
Extreme Obesity	>40	III	Extremely high	Extremely high

*Disease risk for type 2 diabetes, hypertension, and CVD.
†Increased waist circumference can also be a marker for increased risk even in persons of normal weight.

BMI is the most widely used and simple measure of body size and is frequently used to determine the prevalence of overweight and obesity within a population (Ross et al., 1993). However, this measurement does not take into account variations in regional fat distribution and abdominal fat mass. Excess upper body fat is linked with a greater risk of obesity-related morbidity than overall adiposity (Ho et al., 2001). Thus, waist circumference and waist to hip ratio (WHR) appears to be better indicators of visceral adiposity, type 2 diabetes mellitus and CVD than BMI (Haffner et al., 1987). Excess fat accumulation in the intra-abdominal region is referred to as "android obesity" which is most likely to be associated with an altered risk factor profile contributing to CVD and type 2 diabetes, while "gynoid obesity" (lower body obesity located around the hips and buttocks) is seldom associated with metabolic

complications. Therefore, waist circumference is considered an important indicator of abdominal obesity and relative risk of co-morbidities. The waist circumference which is associated with an increased risk of disease is defined as: Men >102 cm (40 in); Women >88 cm (>35 in) (Expert Panel on Detection Evaluation and Treatment of Overweight in Adults, 1998).

Furthermore, BMI and relative disease risk varies among different ethnic populations (World Health Organization, 2000b). Studies have shown that Asians are at an increased risk of developing metabolic syndrome with a lower BMI compared to other ethnic groups (World Health Organization, 2000a). Consequently WHO developed specific BMI criteria for Asians, where a BMI of 23 is considered overweight and a BMI >25 is considered obese (World Health Organization, 2000a). The recommended waist circumference for all ethnic groups is yet to be determined. It is thought that waist circumference may be lower for Asian men compared to Caucasian men and are possibly higher for Pacific Islanders and African Americans. Currently, the limited data available indicates that the risk factors in Aboriginal groups seem to be comparable to those in Asian groups and the risk factors in Torres Strait Islanders seem to be similar to those found in Pacific Islander populations as well (Australian Better Health Initiative, 2008). Table 2.3 shows the ethnic specific values for waist circumference (Australian Better Health Initiative, 2008).

Table 2.3: Ethnic specific values for waist circumference

Country/ethnic group		*Waist circumference (cm)*
Europids	Male	>94
	Female	>80
South Asians (Chinese, Malay & Asian Indian)	Male	>90
	Female	>80
Chinese	Male	>90
	Female	>80
Japanese	Male	>90
	Female	>80
Ethnic South and Central Americans	Use South Asian recommendations until more specific data are available	
Sub-Saharan Africans	Use European data until more specific data are available	
Eastern Mediterranean and Middle East (Arab) populations	Use European data until more specific data are available	

WHR ratio is another useful screening tool to determine upper body fat distribution and potential CVD risk. Males with a WHR >1.0 and females with a ratio of >0.8 have a significantly increased risk of CVD (Dalton et al., 2003). Table 2.2 shows the classification of overweight and obesity by BMI, waist circumference and associated disease risk (Dalton et al., 2003).

2.2.1.2 Prevalence of obesity

According to the Australian Bureau of Statistics, in 2011-2012, there were 63.4 % of Australian adults aged 18 years or over with a BMI in either the overweight or obese range (35 % overweight; 28.3 % obese) (Australian Bureau of Statistics., 2013). Furthermore, there were 35.2 % who were within the normal weight range and 1.5 % were considered underweight. The prevalence of overweight and obesity was higher in men compared to

women (70.3 % versus 56.2 %). Generally, in 2011-2012, overweight and obesity rates increase with age (Australian Bureau of Statistics., 2013). The lowest rates of overweight and obesity are seen in 18-24 year olds, and then peaked for males between the ages of 45-74 and 55-74 for females (Australian Bureau of Statistics., 2013). In 2011-2012, 66 % of females and 60 % of males had a waist circumference indicative of an increased risk to poor health, which showed an increasing trend with age in both males and females (Australian Bureau of Statistics., 2013). National surveys had identified contributing factors to the raise in overweight and obesity rates. The findings from the 1995 National Nutrition Survey, together with the results from 1983 National Dietary Survey of Adults showed a significant increase in energy intake (350 kilojoules or 3-4 % or one slice of extra bread per day) (Cook, 2001).

2.2.1.3 Aetiology of obesity

Body weight regulation involves complicated feedback systems which result in changes in energy intake, appetite and energy expenditure. Even though excess body weight usually occurs as a result of a chronic positive shift of the energy balance equation, as a result of an increase in energy input and a reduction in energy output, the causative factors of overweight and obesity are complex. Dietary intake and physical activity are central to the energy balance equation, however each are influenced directly and indirectly by a complex multifaceted system of determinants, which include social, behavioural, environmental, psychological and genetic factors. The components of energy balance, including energy intake and energy expenditure interact with each other to affect body weight (National Health and Medical Research Council., 2013). The human body attempts to stabilize energy balance via a complex negative feedback system which involves the following hormones: an increase in hunger (e.g. ghrelin), inhibition of energy intake in the short term (e.g. cholecystokinin,

peptide YY, glucagon-like peptide-1), inhibition of energy intake in the long term (e.g. leptin, insulin) and an increase in metabolic rate, and energy expenditure (e.g. triiodothyronine [T3]).

This negative feedback system responds to alterations in body fat and energy stores through modulation of appetite, energy intake and energy expenditure with the aim of maintaining equilibrium in body weight over time (Sumithran et al., 2011). Under normal circumstances, this system defends against weight gain as well as weight loss, however an energy surplus that is large and sustained cannot be maintained, which will therefore result in weight gain (Sumithran et al., 2011). A continuation in weight gain will occur until a new weight results in an increase in energy expenditure and energy balance is re-established (Rosenbaum et al., 2008). This physiological process seeks to maintain energy balance at a higher body weight and will defend against weight loss through an increase in appetite and reducing energy expenditure, if there is an energy deficit (Rosenbaum et al., 2008). Therefore overweight and obesity can result from the upward resetting of the defended level of body weight, rather than the passive accumulation of excess body fat. Factors that affect the physiological control of body weight and energy balance include: A high energy dense diet, large portion sizes of foods and drinks high in fat and sugar (e.g. fast food, soft drinks) and a low intake of low energy foods (vegetables, fruit) and lastly, the adoption of sedentary behaviour and low physical activity levels.

These behavioural and lifestyle factors lead to alterations in adipose tissue structure (e.g. hypertrophy and hyperplasia of adipocytes as well as inflammation) and secretion of hormones (e.g. adipocytokines) (National Health and Medical Research Council., 2013). There are many other complex interactions between biological (e.g. inheritability, epigenetic changes, early life experience), behavioural (e.g. eating and activity habits, stress), social

factors and environmental factors (e.g. food supply, portion sizes, culture, urban design, occupation, disrupted sleep) which are involved in the regulation of energy balance and fat stores (National Health and Medical Research Council., 2013).

2.2.2 Obesity, insulin resistance and the development of metabolic syndrome

The pathogenesis of metabolic syndrome is multifactorial. Several investigations have demonstrated that visceral adiposity is the core predictor of impaired glucose tolerance (Hayashi et al., 2003), insulin sensitivity (Fujimoto et al., 1994, Carey et al., 1996, Cnop et al., 2002, Katsuki et al., 2003, Wagenknecht et al., 2003), dyslipidemia (Pascot et al., 2001, Katsuki et al., 2003, Nieves et al., 2003) and elevated blood pressure (Kanai et al., 1996) which are all components of the metabolic syndrome. Consequently, obesity has been recognised as one of the major causative factors of metabolic syndrome. Insulin resistance has also been considered a potential etiological factor, with many investigators placing a greater importance on insulin resistance rather than obesity in pathogenesis (Reaven, 1988, Ferrannini et al., 1991). It was argued that insulin resistance directly causes other metabolic risk factors. Determining the unique role that insulin resistance plays in the development of metabolic syndrome is complicated by the fact that it is strongly linked to obesity. A rise in insulin resistance is generally observed with the concurrent increase in body fat content (Abbasi et al., 2002). Several studies have shown that visceral adiposity correlates with glucose intolerance with the presence of hyperinsulinemia during an oral glucose tolerance test, depicting an insulin-resistance state (Fujioka et al., 1987). This is evident with an overload of non-esterified fatty acids in the muscle and lipids in the liver, which is linked with an insulin resistant state. It has been shown that free fatty acids compete with glucose for substrate oxidation in the rodent cardiac muscle, thus suggesting that elevated free fatty

acid oxidation induces insulin resistance. A rise in intracellular concentration of free fatty acids results in an increase in intramitchondrial acetyl CoA/CoA and NADH/NAD+ ratios. In turn, pyruvate dehydrogenase is inactivated (Figure 2.3) (Defronzo, 2006). Furthermore, this would then result in an increase in intracellular citrate concentrations and therefore causing the inhibition of phosphofructokinase (a pivotal enzyme involved in glycolysis) (Randle et al., 1963, Petersen and Shulman, 2002a). Subsequently, this gives rise to the accumulation of glucose 6-phosphate which would then lead to the inhibition of hexokinase II activity and finally the rise in intracellular glucose concentration and a decline in glucose uptake.

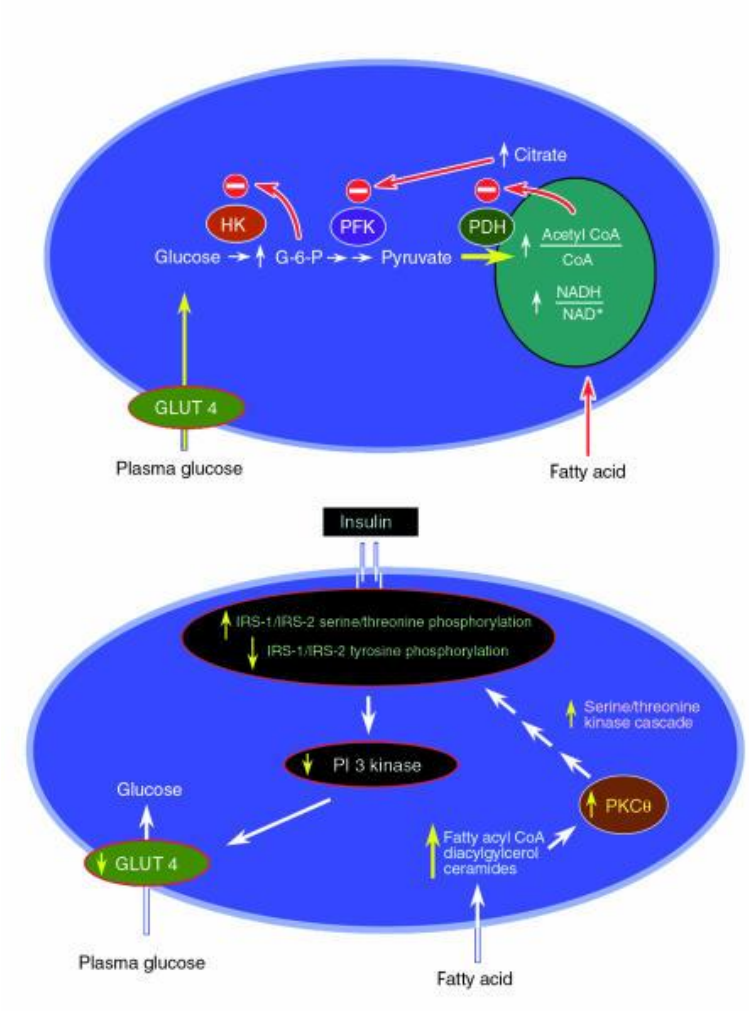


Figure 2.3: Mechanism of fatty acid-induced insulin resistance in the skeletal muscle (Randle et al., 1963); HK, hexokinase II; PFK, phosphofructokinase; PDH, pyruvate dehydrogenase; PKCθ, protein kinase Cθ.

Fatty acids circulate in the blood as triglycerides (lipoproteins) or as non-esterified fatty acids (Abate et al., 1995). Upper body obesity is associated with an increase in free fatty acid levels, which may lead to ectopic lipid accumulation (lipid deposition in non-adipose tissue organs i.e. skeletal muscle) (Petersen and Shulman, 2002b, Yu et al., 2002). Therefore, saturation of adipose tissue and ectopic fat storage in metabolically active tissues known to be highly insulin-responsive i.e. skeletal muscle (Heilbronn et al., 2004) results in lipotoxicity (lipid induced metabolic damage; lipid uptake exceeds capacity of lipid storage) and altered secretion of adipocytokines (Carr et al., 2004). These cascading events are thought be the major underlying mechanisms of metabolic syndrome.

It has been demonstrated that adipose tissue is not just simply an inert storage depot for lipids. Adipocytes are known to synthesize and secrete several biologically active proteins into the blood circulation. These bioactive proteins are referred to as "adipocytokines" (adipocyte-derived hormones). Intra-abdominal fat is metabolically active as a source of adipocytokines. Secretory proteins collectively named adipocytokines include leptin (Yun et al., 2010), adiponectin (Yatagai et al., 2003, Cnop et al., 2003), plasminogen activator inhibitor type 1 (PAI-1) (Alessi et al., 1997, Giltay et al., 1998) tumour necrosis factor alpha (TNF-α) (Katsuki et al., 1998, Bertin et al., 2000, van Harmelen et al., 2002), non-esterified fatty acids (Abate et al., 1995), interleukin 6 (IL-6) (Fontana et al., 2007) and many others.

Circulating leptin is a hormone secreted by adipocytes as a product of the obese (ob) gene that reflects the body's fat content (Zhang et al., 1994). Circulating leptin increases in response to saturation of adipose tissue (de Luis Roman et al., 2006, Keim et al., 1998, Heini et al., 1998, Yun et al., 2010). Genetic deficiency of leptin in rodents (Caro et al., 1996) and humans (Montague et al., 1997) leads to hyperphagia and obesity. Several studies have demonstrated that in humans leptin circulates in direct proportion to adipose tissue mass

(Maffei et al., 1995, Considine et al., 1996, Ostlund et al., 1996, Havel et al., 1996). Human obesity is characterized by raised leptin levels, indicative of a leptin-resistant state in obese patients (Considine et al., 1996, de Luis Roman et al., 2006). Leptin mRNA expression and protein levels are much higher in obese than lean individuals (Maffei et al., 1995). Therefore, circulating leptin plays a crucial factor in the development of obesity (Yun et al., 2010).

Adiponectin is one of the most abundant adipose tissue specific adipocytokines. Adiponectin has been demonstrated to be in lower levels in individuals with obesity (Arita et al., 1999), type 2 diabetes (Hotta et al., 2000), insulin resistance (Weyer et al., 2001), dyslipidemia (Matsubara et al., 2002a), coronary heart disease (Ouchi et al., 1999) in addition to peripheral resistance to adiponectin in skeletal muscle (Mullen et al., 2009). Low levels of plasma adiponectin have also been inversely associated with BMI (Arita et al., 1999). With a reduction in body weight in obese individuals, plasma adiponectin concentrations have been found to increase, therefore indicating that obesity related hypoadiponectinemia is reversible (Hotta et al., 2000).

Studies have shown that adiponectin is capable of enhancing insulin sensitivity, increasing fatty acid oxidation and glucose uptake, as well as suppressing hepatic glucose production (Wu et al., 2003, Berg et al., 2001, Combs et al., 2001, Fruebis et al., 2001, Yamauchi et al., 2001). These studies suggest that adiponectin acts through multiple tissues to enhance insulin sensitivity therefore adiponectin has insulin sensitizing properties.

Adiponectin has been shown to reduce or inhibit TNF-α signalling pathway in cultured human aortic endothelial cells by inhibiting the expression of intercellular adhesion molecules, vascular cell adhesion molecules and E-selectin in endothelial cells (Ouchi et al., 1999). Adiponectin has also been shown to reduce lipopolysacharide TNF-α production in cultured macrophages by suppressing phagocytic activity (Yokota et al., 2000). Therefore

these studies suggest that adiponectin may have anti-inflammatory and anti-atherogenic properties (Matsubara et al., 2002b, Yang et al., 2001).

TNF-α has been identified as a proinflammatory adipocytokine expressed in adipose tissue (Hotamisligil et al., 1993). Studies have demonstrated that TNF-α may induce insulin resistance and is suggested to represent a link between obesity and insulin resistance (Hotamisligil et al., 1993). Evidence shows that TNF-α promotes insulin resistance by the inhibition of the insulin receptor substrate 1 signalling pathway (Hotamisligil et al., 1996). The expression of TNF-α in adipose tissue is greatly induced by obesity in both human and animal studies (Hotamisligil et al., 1995, Hotamisligil et al., 1993). The degree of obesity and level of insulin resistance is strongly correlated with an increase in TNF-α mRNA expression in obese human and animal model studies (Kern et al., 2003, Kern et al., 2001).

IL-6 is another proinflammatory adipocytokine secreted by adipose tissue. Elevated levels of IL-6 have also been strongly associated with insulin resistance (Vozarova et al., 2001). The risk of developing type 2 diabetes increases significantly with increased levels of IL-6 (Pradhan et al., 2001). Following weight reduction in obese individuals, IL-6 levels in serum and subcutaneous adipose tissue are significantly decreased (Bastard et al., 2000).

Elevated circulating levels of PAI-1 have reportedly been shown to be linked with obesity (Mavri et al., 1999, McGill et al., 1994). PAI-1 is positively linked with BMI in both women (Landin et al., 1990) and men (Urano et al., 1993). PAI-1 is also associated with other anthropometric measures of obesity including WHR, reflecting central obesity and with several metabolic abnormalities such as insulin and plasma triglycerides (Juhan-Vague et al., 1987). With weight reduction, there is a significant decrease in plasma PAI-1 which is strongly correlated with changes in adipose tissue (Mavri et al., 1999).

Different adipose tissue compartments may be associated with differential metabolic risk (Poirier and Despres, 2003). Excess upper body fat can accumulate either as subcutaneous adipose tissue or visceral adipose tissue (Fox et al., 2007). Subcutaneous adipose tissue can be separated into a deep and superficial layer by fascia. Visceral fat and deep subcutaneous fat are metabolically active while superficial adipose tissue is comparatively inert metabolically (Sniderman et al., 2007). Therefore visceral fat and deep subcutaneous fat provide a greater link with metabolic syndrome.

An increase in body weight leads to an increased rate of lipolysis in abdominal fat depots, which results in an increase in non-esterified fatty acids (Nicklas et al., 1996). An increase in non-esterified fatty acids in the liver leads to an increase in hepatic gluconeogenesis and lipoprotein production as a result of hepatic insulin resistance and an increase in very low density lipoprotein (VLDL) cholesterol (Rendell et al., 2001). Consequently, abnormalities including dyslipidemia and hepatic steatosis (non-alcoholic fatty liver disease) may occur (Misra and Vikram, 2003). Therefore, there are several factors involved in the pathogenesis of metabolic syndrome and associated manifestations, with central obesity and insulin resistance as the core contributing factors of the condition.

2.2.3 Dyslipidemia and metabolic syndrome

Dyslipidemia is an important component of metabolic syndrome, characterized by elevated total cholesterol and triglycerides and low high density lipoprotein (HDL) cholesterol and high low density lipoprotein (LDL) cholesterol levels. Abnormalities in blood lipid profile can increase the risk of developing CHD and cardiovascular diseases (Brewer, 1999).

LDL particles are major carriers of cholesterol, transporting 60 % of total circulating cholesterol. LDL cholesterol is derived from VLDL cholesterol and transports cholesterol to peripheral tissues. Elevated levels of LDL in the blood contribute to the initiation and progression of arterial plaque which can lead to the development of atherosclerosis (Thomas, 2001). HDL are small dense particles derived from chylomicron hydrolysis, and are composed primarily of protein and transport cholesterol from cells back to the liver (Thomas, 2001, Whitney E & Rolfes S, 2008). HDL is involved in the removal of unesterified cholesterol and other lipoproteins from cells where it may have built up and then is returned to the liver for excretion. Epidemiological studies have identified a strong inverse relationship between high HDL cholesterol and CHD (Assmann et al., 1996b). It has been established that cholesterol and other lipoproteins are transported from peripheral cells in an esterified form back to the liver, in turn reducing cholesterol deposition in the vascular endothelium. HDL particles may provide other beneficial properties including anti-oxidative, anti-inflammatory, anti-atherosclerotic and anti-thrombotic properties (Chapman, 2006). Dysfunction of HDL is an independent pro-atherogenic factor.

Triglycerides are found in VLDL cholesterol and elevated levels of triglycerides are associated with an increased incidence of coronary events (Assmann et al., 1996a). A study conducted by Hokanson et al (1996), suggested that for every 1 mmol/L increase in serum triglyceride concentration, the relative risk of CHD increased by 37 % in women and 14 % in men (Hokanson and Austin, 1996).

The link between dyslipidemia and obesity is associated with an increase in triglyceride level. Abdominal fat exhibits increased lipolytic activity, chiefly in the visceral depot which has been shown to affect insulin action and the disposal of glucose through a rise in non-esterified fatty acids (Griffin et al., 1999). This increase in free fatty acids in the visceral

depot is then drained into the portal circulation, therefore affecting hepatic insulin sensitivity thereby resulting in an increase in hepatic glucose production. In turn the increase in free fatty acids can lead to the ectopic accumulation of triglycerides in the liver and muscle (Ginsberg, 2000).

An influx of free fatty acids into the liver causes a rise in hepatic triglyceride content in obese individuals, which increases the likelihood of developing dyslipidemia. Hepatic triglyceride overload is the result of an elevation in serum triglycerides. In turn, the liver secretes VLDL into the blood stream which often occurs with an altered lipid turnover in peripheral tissues (Lemieux et al., 2000).

Visceral adiposity has been shown to be significantly associated with lower HDL cholesterol (Pascot et al., 2001), higher triglycerides levels (Ribeiro-Filho et al., 2003) and higher LDL cholesterol (Pascot et al., 2000). A study conducted by Chehrei et al., (2007) observed a significant correlation between dyslipidemia and central obesity using waist circumference and waist to height ratio as predictors of metabolic risk (Chehrei et al., 2007).

2.2.4 Non-alcoholic fatty liver disease

Non-alcoholic fatty liver disease (NAFLD) is characterized by fatty infiltration of liver cells (simple steatosis), fatty liver accompanied by inflammation (steatohepatitis) and end stage liver disease (Brea et al., 2005). NAFLD is strongly linked with visceral obesity, insulin resistance, type 2 diabetes, hypertriglyceridemia, and reduced HDL cholesterol (Marchesini et al., 2001). NAFLD is considered a hepatic manifestation of metabolic syndrome which includes several pathologies such as steatosis, inflammation, fibrosis and cirrhosis of the liver (Riley et al., 2007).

Contributing factors involved in the development and progression of NAFLD include insulin resistance, oxidative stress, excess accumulation of triglycerides in hepatocytes and inflammatory adipocytokines (Riley et al., 2007). However, the specific mechanisms underlying the cause of the disease are yet to be fully elucidated. Studies have established a correlation between fatty liver disease and risk factors of metabolic syndrome (Hsiao et al., 2007). Also evidence suggests that NAFLD can exacerbate CVD risk independent of the metabolic syndrome (Targher et al., 2005).

γ-glutamyltransferase (GGT) and serum alanine aminotransferase (ALT) concentrations are known markers of hepatocyte injury and have been shown to be associated with liver lipid content, insulin resistance and histological features of NAFLD (Vozarova et al., 2002, Dixon et al., 2006). Higher levels of ALT are linked with a decrease in hepatic insulin sensitivity and the incidence of type 2 diabetes (Hanley et al., 2004).

Recent findings have shown an associated between NAFLD and carotid artery intima-media thickness, a marker of early stage atherosclerosis (O'Leary and Polak, 2002, Brea et al., 2005). Evidence suggests that hepatic lipid build-up is atherogenic beyond its link with insulin resistance (Brea et al., 2005). The mechanisms behind NAFLD and accelerated atherosclerosis are poorly understood. Many studies have documented that insulin resistance predicts CVD and plays a major role in the development of poor clinical outcomes in patients with NAFLD (McCullough, 2004). Advanced NAFLD may act as a stimulus for enhancing insulin resistance and dyslipidemia, resulting in accelerated atherosclerosis (Targher et al., 2005). These findings are confirmed in recent work demonstrating that increased liver enzymes independently predict metabolic syndrome development (Hanley et al., 2005).

The mechanism linking carotid intima-media thickness and NAFLD might be associated with subclinical inflammation and elevated oxidative stress, which are said to be causative factors

involved in the progression of simple steatosis to NAFLD in more advanced forms (McCullough, 2004). The presence of reactive oxygen species (ROS) as a result of steatosis-stimulated fatty acid oxidation, cytokine release, hepatocyte injury and a proinflammatory state is likely to be responsible for the liver damage seen in NAFLD and cause further atherogenic stimuli to the already high proinflammatory/oxidative state associated with metabolic syndrome (Eckel et al., 2005).

2.2.5 Atherosclerosis, inflammation and metabolic syndrome

Many features of metabolic syndrome such as visceral obesity are linked with a low-grade inflammatory state (Paoletti et al., 2006). Evidence suggests that inflammatory cytokines and endothelial dysfunction (as outlined in Figure 2.4) are associated with metabolic syndrome, which has led to the development of an inflammatory-based causal pathway (Maury and Brichard, 2010).

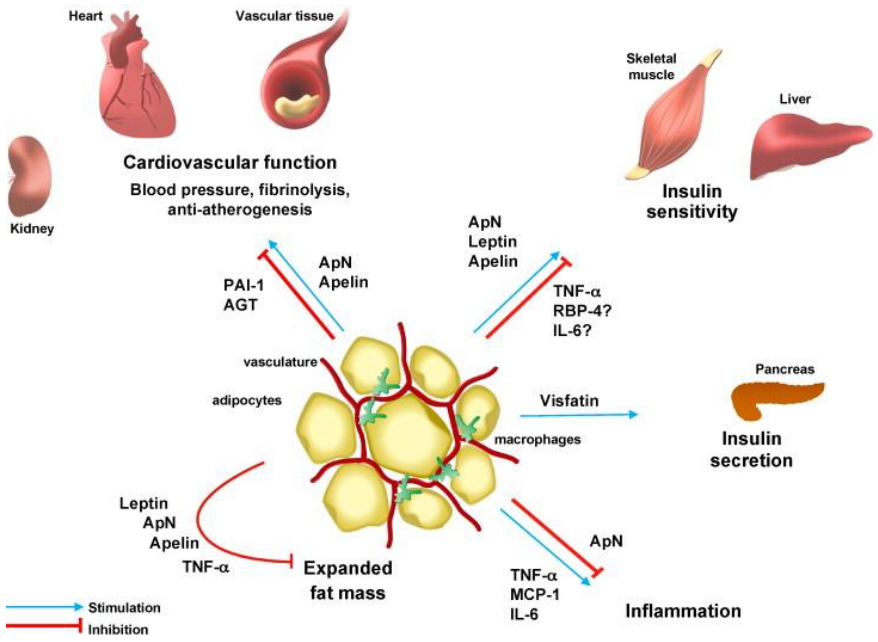


Figure 2.4: Adipokines involved in the pathogenesis of metabolic syndrome (Maury and Brichard, 2010).

Metabolic syndrome is thought to be the first order risk factor for atherothrombotic complications. Metabolic syndrome is linked with increased carotid intima-media thickness in adults (Skilton et al., 2007). It has been demonstrated that there is a significant relationship between increased LDL levels and carotid atherosclerosis in metabolic syndrome (Montalcini et al., 2005). LDL cholesterol is a strong predictor of CHD in patients with diabetes and insulin resistance (Howard et al., 2000). Furthermore, individuals with risk factors linked with insulin resistance and high LDL cholesterol were related to an excess of carotid atherosclerosis (Golden et al., 2002). This finding suggests that high levels of LDL cholesterol play an important role in the pathogenesis of atherosclerosis.

This lipoprotein is a strong predictor of oxidized LDL (oxLDL) levels, which is a key mediator in atherosclerosis (Montalcini et al., 2005). Oxidation of LDL is a hallmark of the development of atherosclerosis (Witztum, 1994). Circulating levels of oxLDL and C-reactive protein (CRP) are associated with excess abdominal fat (defined by waist circumference), which are known risk factors of atherosclerosis (Weinbrenner et al., 2006, Ridker, 2003, Barinas-Mitchell et al., 2001).

Uric acid is also a biomarker for atherosclerosis. Although uric acid is not part of the metabolic syndrome definition, many epidemiological studies have observed associations between elevated levels of serum uric acid levels and CVD risk and metabolic syndrome (Kawamoto et al., 2006). Increase uric acid may induce endothelial dysfunction, inflammation and insulin resistance (Kanellis and Kang, 2005). Elevated uric acid is also associated with BMI, hypertension and dyslipidemia (Ishizaka et al., 2005).

In addition, hyperhomocysteinemia is a cardiovascular risk factor that has been shown to result in endothelial dysfunction. This has been identified in animal models with hyperhomocysteinaemia (Virdis et al., 2003). Individuals with severely high levels of

homocysteine may present with a rare genetic disorder referred to as homocystinuria which is linked with early arteriosclerosis (Malinow, 1995). Severe hyperhomocysteinaemia is an important atherosclerotic arterial disease and can also lead to hepatic steatosis (Ferre and Foufelle, 2007).

Microalbuminuria is also a predictor of cardiovascular disease and mortality. Studies have shown that microalbuminuria is associated with central obesity and hypertension (Palaniappan et al., 2003). It has been suggested that microalbuminuria is involved in the development of vascular endothelial damage by increasing vascular permeability and therefore can be seen as an early indicator of atherosclerosis (Palaniappan et al., 2003).

Various components of metabolic syndrome are related to specific inflammatory markers including CRP, white cell count and fibrinogen. For instance, increasing levels of CRP is associated with abdominal obesity (increased waist circumference), BMI, dyslipidemia, hypertension and insulin resistance (Festa et al., 2000). Highly sensitive CRP plasma levels are elevated in patients with insulin resistance, obesity and are predictors of diabetes and CHD (Festa et al., 2000). Furthermore, CRP is used as an indicator tool to predict the occurrence of future cardiovascular events (Clearfield, 2005).

The adipocytokines including leptin, TNF-α, PAI-1, IL-6 and angiotension contribute to vascular inflammation and oxidative stress (Dandona et al., 2005). Leptin has been shown to have anti-fibrinolytic and procoagulant properties. Also leptin is involved in thrombus formation and atherosclerosis through the amplification of vascular inflammation and proliferation, while adiponectin promotes insulin sensitivity and inhibits inflammation (Nieuwdorp et al., 2005). Adiponectin has also been found to decrease the endothelial expression of vascular cell adhesion molecule-1(VCAM-1), intercellular adhesion molecule-1 (ICAM-1), P-selectin, reduces macrophage phagocytic activity and inhibits foam cell

formation in vitro (Yokota et al., 2000). In contrast, resistin is expressed in macrophages and correlates with inflammatory markers, increases the gene expression of ICAM-1 and VCAM-1 whilst enhancing monocyte chemoattractant protein-1 (Verma et al., 2003). Thus, resistin and adiponectin interfere with monocyte adherence to vascular endothelium, thereby stimulating monocytic migration to the subendothelial space, which is a major event involved in the development of atherosclerosis.

2.2.6 Hypertension and metabolic syndrome

High blood pressure is a well-recognized component of metabolic syndrome and by itself an important risk factor for cardiovascular disease. Hypertension appears to be the strongest predictor of cardiovascular mortality out of all the components of metabolic syndrome. The pathological mechanisms of hypertension in metabolic syndrome are not fully understood. Hypertension in metabolic syndrome has multifactorial causes with the following mechanisms thought to play a pivotal role in the pathophysiology of metabolic syndrome's hypertension, these include: sympathetic hyperactivation, increased renin-angiotensin-aldosterone activity and endothelial dysfunction (Mendizabal et al., 2013).

Hyperinsulinemia, hyperleptinemia and hyperlipidemia are the three main conditions typical of the metabolic syndrome that may lead to the exacerbation of sympathetic tone. It has been reported that hyperinsulinemia causes a considerable rise in circulating noradrenaline concentrations accompanied by an elevation in blood pressure (Rowe et al., 1981). Furthermore, high levels of insulin increase sodium reabsorption which may lead to expansion of extracellular fluid volume thereby predisposing hypertension (Vierhapper, 1985).

In addition, obesity impairs renal-pressure natriuresis and causes sodium retention. Arterial pressure is elevated in obese subjects in order to maintain sodium balance, which indicates renal-pressure natriuresis (Hall, 1997). According to the Framingham study, risk estimates show that approximately 80 % of hypertension in men and 65 % in women can be attributed to obesity (Garrison et al., 1987). There is a clear link observed between arterial pressure and BMI. The prevalence of obesity has increased dramatically in the past decade which has given rise to the prevalence of hypertension globally (Sharma and Chetty, 2005). Hypertension is associated with moderate weight gain, however there is variability in the blood pressure response to weight gain, therefore hypertension may not occur in all obese individuals (Bjorntorp, 1987). For example, the North American Pima Indians have a high prevalence of obesity, however they do not have the corresponding high incidence of hypertension (Berchtold et al., 1981).

Furthermore, there is a link between elevated circulating leptin levels, obesity and an increase in sympathetic activity. High levels of circulating leptin are associated with an increase in renal sympathetic tone seen in obese subjects (Eikelis et al., 2003). Elevated leptin concentrations in obese subjects elicit endothelial dysfunction by nitrogen oxide (NO)-dependent vasodilation as well as causing impairment of endothelial-dependant relaxation, thus producing endothelial dysfunction (Knudson et al., 2005). Also, high levels of circulating levels of free fatty acids in central obesity is associated with an increased sympathetic nerve outflow (Alvarez et al., 2002).

High levels of circulating adiponectin have been found to carry out a preventable role in the development of vascular changes by improving NO-dependent vasodilation via opening voltage-dependent potassium channels (Fesus et al., 2007). While low concentrations of

adiponectin are associated with insulin resistance, thus resulting in vascular changes which could provide a background into hypertension.

It has also been demonstrated that insulin is involved in the stimulation of endothelin-1 (vasoconstrictor) gene expression in endothelial cells (Oliver et al., 1991). It was then shown that insulin modulates circulating endothelin-1 levels (Wolpert et al., 1993) and high levels of plasma endothelin-1 have been observed in subjects with diabetes (Ferri et al., 1995). It is proposed that hyperinsulinemia observed in insulin resistance causes an elevation in sodium reabsorption and sympathetic activity, thereby increasing arterial pressure. To support this hypothesis, there is evidence of a corelation between high blood pressure levels and insulin resistance (Modan et al., 1985) and essential hypertension has been asserted as an insulin resistance state (Ferrannini et al., 1987).

The renin-angiotensin-aldosterone system (RAAS) plays an imperative role in controlling blood pressure through the modulation of vascular tone and renal function. There is evidence of an increase in RAAS activity in subjects with metabolic syndrome, thus contributing to obesity-induced hypertension. For instances, plasma renin activity, and the production of angiotensin II are elevated in obese subjects (Hall, 1997). Furthermore, expression of angiotensinogen is higher in intra-abdominal fat, which would explain the elevated circulating concentrations observed in obesity (Boustany et al., 2004). Aldosterone is closely related to the physiology of angiotensin II, which has been found to be elevated in the majority of obese hypertensive subjects with central adiposity (Goodfriend and Calhoun, 2004). In addition, increased levels of aldosterone are induced by hyperinsulinemia which could provide a potential mechanism for the development of complications seen in hypertensive obese subjects (Rocchini et al., 1990).

SECTION III: A REVIEW ON THE EFFICACY OF CARALLUMA FIMBRIATA EXTRACT AND CITRUS SINENSIS EXTRACT IN THE TREATMENT OF METABOLIC SYNDROME

2.4.1 Background

Excess central adiposity and its concomitant health risks are amongst the most common health conditions managed by allied health professionals. Effective strategies are needed to intervene in the development and progression of obesity and associated metabolic disorders. The use of botanical extracts for weight loss has become a rapidly growing therapeutic area, which is widely embraced by the general public. However, even though there are many beneficial effects of botanical extracts with anti-obesity potential reported in the literature (Santos et al., 2010), there are also several limitations associated with using botanical extracts as therapeutic agents that should be mentioned. These may include the following (Fabricant and Farnsworth, 2001):

1. The active constituent/ingredients are often unknown or only partly explained.
2. Adverse effects from plant extracts are not well documented in the literature.
3. It is difficult to relate one class of phytochemicals to specific biological targets.
4. Plants as biologic systems have inherent potential variability in their chemistry and resulting biologic activity.
5. Botanical supplements are usually mixtures of many constituents.
6. The source and quality of the raw material are variable.

Botanical extracts in combination with lifestyle modification may be effective agents for attenuating the development of metabolic syndrome as they often comprise of a vast range of bioactive compounds that possess multiple mechanisms of action that may potentiate each other's activity or elicit a synergistic effect, enabling a greater benefit than that of a single chemical entity (Graf et al., 2010).

Furthermore, the use of these botanical extracts is often attributed to appetite suppressing properties, which may act by causing a reduction in hunger and energy intake thereby facilitating significant changes in body composition. Therefore, it is possible that the complexity of this metabolic disorder "metabolic syndrome" may be addressed with a treatment strategy comprising of a combination of these complex compounds that possess appetite suppressing and anti-obesity properties. Commonly available botanical extracts with potential appetite suppressing and anti-obesity properties were critically reviewed (Astell et al., 2013c, Astell et al., 2013b). Please refer to Appendix 1 and Appendix 2 for full publications.

Despite the widespread use of putative botanical supplements with anti-obesity, anti-diabetic, anti-hypertensive and lipid lowering benefits, few botanicals have been evaluated effectively in randomised double blind placebo controlled clinical trials. This thesis will focus on the effectiveness of two botanical supplements, chiefly *Caralluma fimbriata* and *Citrus sinensis* (Moro variety) for the treatment of metabolic syndrome.

2.4.2 *Caralluma fimbriata* extract

2.4.2.1 Description of C. fimbriata

The Genus *Caralluma* belongs to the Apocynaceae family under the subfamily Asclepiadoideae (Milkweed family) (Pellati et al., 2002). The Apocynaceae family is in the major group Angiosperms (Flowering plants). The Apocynaceae family is composed of approximately 260 species that are grouped into three subgenera: *Caralluma* subgen. *Boucerosia* (Wight & Arn.) M.G.Gilbert, *Caralluma* subgen. *Desmidorchis* (Ehrenb) M.G.Gilbert, and *Caralluma* subgen. *Urmalcala* M.G.Gilbert (Pusztai et al., 1995). Previously, the *Caralluma* genus was placed in the Asclepiadaceae family, however, it has since been merged into the Apocynaceae family (Pellati et al., 2002).

The Genus *Caralluma* comprises of approximately 70 species and was first named by Brown R. (1810) to illustrate an Indian species *Caralluma adscendens* with an especially characteristic elongated flowering succulent stem. Many variations of *C. adscendens* have been acknowledged in India and Sri Lanka, including var. *attenuata* (Wight 1848) Gravely & Mayuranathan (synonymous name: *Caralluma attenuata*) and var. *fimbriata* (Wallich 1830) Gravely & Mayuranathan (synonymous name: *Caralluma fimbriata*). *Caralluma subulata* (Forssk. ex Decne. 1938) from the Arabian Peninsula and *Caralluma dalzielii* (N.E.Br. 1812) from the Sahelian zone in Africa east to Somalia have been placed into synonymy with *Caralluma adscendens* var. *adscendens* a long time ago (Grant et al., 1993).

In western India, this perennial herb has many local names, which include: *Ranshabar, Makad shenguli, Shindala makadi, Kullee Mooliyan, Karallamu, and Yungmaphallottama* (Donatucci et al., 1987).

2.4.2.2 Morphology of C. fimbriata

C. fimbriata is a small shrub that has succulent stems (30-60 cm tall, 2 cm diameter) with erect branches growing basally. The recumbent stems are concavely 4-angled to the apex narrowing to a pointed tip. The tubercles are blunt and protruding, extending upwards and horizontally with reddish dots and milky white sap (latex) is found. The angled stems are covered with spines that are actually small caducous leaves. Rudimentary, the leaves of the succulent are small and simple (Grant et al., 1993). One or two flowers can be found on a stem and are situated axillary and scattered. The flower has both stamens and carpels therefore it is bisexual or a "perfect" flower. The fleshy flowers are star-shaped (5-merous), regular, drooping and emit a fetid smell (Figure 2.5). These foul smelling flowers are some of the worst smelling succulents (Grant et al., 1993).

Figure 2.5 Flower of *C. fimbriata*

The stalk supporting the flower (Pedicel) is 1-4mm long and the sepals are triangular (2-3 mm long) and sharply pointed (acute) (Figure 2.6). The petals of the flower (Corolla) are 2.5 cm in diameter, flat and bell-shaped (Campanulate). The flowers are pale green with small purple dots that are at times striped. The corolla lobules (1-3 cm x 1-1.5 cm) are oval shaped,

lanceolate, bluntly acuminate, horizontally striped and basally broader, with the apex having long hairs and is brownish to red in colour. The outer corona or crown is bowl-shaped, with the lobes brown to dark purple in colour, while the inner corona lobes are longer, thread-like and deeply divided. The fruit consists of a pair of fusiform follicles (10-15 cm x c. 1 cm) and apex acuminate. The seeds are oblong (c. 12mm x c. 4mm) with a turf of white hairs (3-4 cm long) on the apex of the seed. The flowers are pollinated and greatly attract flies (Grant et al., 1993).

Figure 2.6 Stem of *C. fimbriata*

2.4.2.3 Origin, propagation, planting, harvesting, ecology and distribution of C. fimbriata

The tender succulent plant flourishes wild in India, Pakistan, the Canary Islands, Arabia, southern Europe, Sri Lanka and Afghanistan (Donatucci et al., 1987). The *Caralluma* plant is distributed in the dry regions of tropical Asia, central, northern eastern Africa and the southern Mediterranean (Pellati et al., 2002). *C. fimbriata* is the most prevalent of the *Caralluma* genus as it is planted as a roadside shrub and boundary marker in gardens in Andhra Pradesh, Karnataka, and Tamil Nadu of India and grows wild in urban centres. The

shrub can be easily propagated using the stem cutting method. Plant regeneration of *C. fimbriata* by in-vitro culture of nodal explants has been achieved. Harvesting of *C. fimbriata* can be undertaken throughout the year. *C. fimbriata* is found on rocky hills and gravelly soils, and at sea level to 1000m altitude. The succulent can tolerate an annual rainfall at a minimum of 400mm and high temperatures.

2.4.2.4 Ethnobotanical uses of C. fimbriata

Traditionally, the edible plant has been used for many centuries in native Indian diets with claims in folklore of hunger suppressing activity. *C. fimbriata* is known as the "Indian Hoodia" and is listed as a vegetable in the Indian Health Ministry's comprehensive compilation on medicinal plants and the Wealth of India (1992). Testimonials from individuals have stated that it is often used as a vegetable, eaten raw or cooked with spices in dry rural India. The fruit of the plant is sometimes eaten cooked and eaten with added salt. For example, the culinary herb is consumed daily as a vegetable in the Kolli Hills of South India. The cactus plant is also preserved as chutneys and pickles in the arid regions of Andhra Pradesh. In Western India, the edible plant is accepted as a famine food and is consumed by boiling and salting the green follicles of the succulent.

When no food is available in these arid and semiarid regions, *C. fimbriata* is eaten as a substitute for food. South Indians are known to chew chunks of *C. fimbriata* to suppress appetite and to boost endurance when on a day's hunt. These Indian tribesmen make their living as hunters, foragers, wood collectors and plant collectors. When hunting in the forest, they do not take any food with them, so to ensure they have enough stamina and don't get hungry throughout the day they chop off the stem of the succulent and chew a handful at a

time. This quenches the tribesmen's thirst and reduces their hunger, while maintaining energy levels.

2.4.2.5 Chemical constituents of C. fimbriata

The key phytochemical ingredients in *C. fimbriata* include pregnane glycosides, saponin glycosides and bitter principles (Bader et al., 2003). The appetite suppressing properties of *C. fimbriata* could be attributed to the pregnane glycosides, which are a rich source in the *Caralluma* genus (Kunert et al., 2008). Eleven pregnane glycosides, 2-7 and 9-13 have been isolated from the plant extract with four (compounds 10-13) containing a novel genin (Kunert et al., 2008). The pregnane glycosides isolated from the aerial parts of the plant include: caratuberside A-B, bouceroside I-X, tomentogenin, luteolin-4'-O-neohesperidoside and kaempferol-7'-O-neohesperidoside.

2.4.2.6 Mechanism of action of C. fimbriata

The anorectic properties of *C. fimbriata* extract have been reviewed and found to have a similar mechanism of action to another botanical compound known as *Garcinia cambogia* (Preuss et al., 2004a). The mechanism of action behind *G. cambogia* has been shown to help aid weight loss in short term clinical trials (Marquez et al., 2012). Evidence-based research on the mechanisms of *G. cambogia* may give an indication as to how *C. fimbriata* works to reduce weight. The main phytochemical constituent in *G. cambogia* is hydroxycitric acid (HCA). Supplementation with HCA has been shown to result in weight loss in humans without stimulating the central nervous system (Preuss et al., 2004b). HCA is a competitive inhibitor of adenosine triphosphate (ATP)-citrate lyase, an extramitochondrial enzyme

involved in the initial steps of de novo lipogenesis (Preuss et al., 2004b). Accordingly, HCA reduces the transformation of citrate into acetyl-coenzyme A, a step required for the formation of fatty acids in the liver. It is postulated that the pregnane glycoside blocks the activity of citrate lyase enzyme, resulting in the inhibition of fatty acid biosynthesis. Moreover, it also blocks the formation of malonyl-coenzyme A, thus enhancing stored fatty acid oxidation.

G. cambogia extract mechanism of appetite suppression is similar to that of *C. fimbriata*. Appetite suppressing properties of *C. fimbriata* are believed to be a secondary effect on the appetite control centre of the brain (Preuss et al., 2004b). It has been established that *G. cambogia* supplementation results in the reduction of food intake in animals, suggesting that the botanical has anti-obesity properties, and it has been revealed that the availability of serotonin in isolated rat brain cortex is increased, which may influence satiety (Sullivan et al., 1974a, Jena et al., 2002, Lowenstein, 1971, Ohia et al., 2001, Ohia et al., 2002, Sullivan et al., 1974b, Triscari and Sullivan, 1977).

It is believed that the pregnane glycosides in *C. fimbriata* inhibit the hunger sensory mechanisms of the hypothalamus. However, it is uncertain as to how the pregnane glycosides may suppress appetite; it is thought that the pregnane glycosides amplify the signalling of energy sensing function in the hypothalamus (MacLean and Luo, 2004). Another hypothesis is that *C. fimbriata* may down-regulate ghrelin synthesis in the stomach and neuropeptide-Y in the hypothalamus, resulting in appetite suppression (Gardiner et al., 2005). *H. gordonii* has been reported to have a similar appetite suppressing action, in which a steroidal glycoside was isolated, which verified anorectic activity in animals (Kuriyan et al., 2007). A phytochemical profile of the plant extract revealed structural similarities with another

appetite suppressant *H. gordonii*. It is evident that the steroidal saponins isolated from *C. fimbriata* are similar in structure to P57AS3, which was isolated from the slow-growing succulent plant *H. gordonii* (Van Heerden and P.J., 2007). However, cellular and molecular targets of the pregnane glycosides or the examination of the specific mechanisms of action on how the pregnane glycosides actually affect appetite were not examined.

Another potential anti-obesity botanical known as *Asclepias incarnata* is also from the milkweed family like *C. fimbriata* and *H. gordonii*, and is enriched with pregnane glycosides (Komarnytsky et al., 2013). A recent study conducted by Komarnytsky et al (2013) investigated the potential of pregnane glycoside-enriched extract from swamp milkweed roots (*A. incarnatin*) to reduce food intake (Komarnytsky et al., 2013). A significant reduction in spontaneous and fasting-induced food consumption in Wistar rats was linked with *A. incarnata* oral administration which was observed in combination with a rise in gastric accommodation and delay of gastric emptying (Komarnytsky et al., 2013).

Komarnytsky et al (2013) also revealed the appetite-regulatory effects of treatment with the major pregnane glycoside constituent of the swamp milkweed named ikemagenin (12β-cinnamoyl-3,8,12,14β-tetrahydroxypregn-5-en-20-one). This study demonstrated that the swamp milkweed, an alternative abundant source of biologically active pregnane glycosides are rapidly transmitted to central tissues chiefly the hypothalamus, in which the expression of orexigenic and anorexic neuropeptides become affected. The animals were treated with ikemagenin (10 mg/kg BW) following an overnight fast and food intake was then measured three hours after the gavage of treatment (Komarnytsky et al., 2013). The rodents were then sacrificed and the hypothalamus was collected and underwent processing for RNA and protein extraction. Cell culture studies were also performed using the rat glioma C6 cell line

which expresses brain-derived neurotropic factor (BDNF) (central peptide that decrease food intake & body weight gain) (Komarnytsky et al., 2013). It was found that Ikemagenin exerted its appetite-regulating effect via triggering a decline in hypothalamic AgRP mRNA levels by 0.6-fold (orexigenic peptide) and caused a 1.4-fold rise in the BDNF mRNA levels without inflicting an effect on the anorectic peptides pro-opiomelanocortin (POMC) and Cocaine and amphetamine-regulated transcript (CART) mRNA (Komarnytsky et al., 2013). AgRP is a fundamental part of the melanocortin system involved in energy balance and food intake regulation by antagonistic effects on melanocortin 3 and 4 receptors that trigger an ongoing increase in food consumption (Cone et al., 2001). The results from this study conducted by Komarnytsky et al (2013) demonstrate that pregnane glycosides have many effects on food intake which may be mediated by hypothalamic neuropeptides (Figure 2.7) (Komarnytsky et al., 2013).

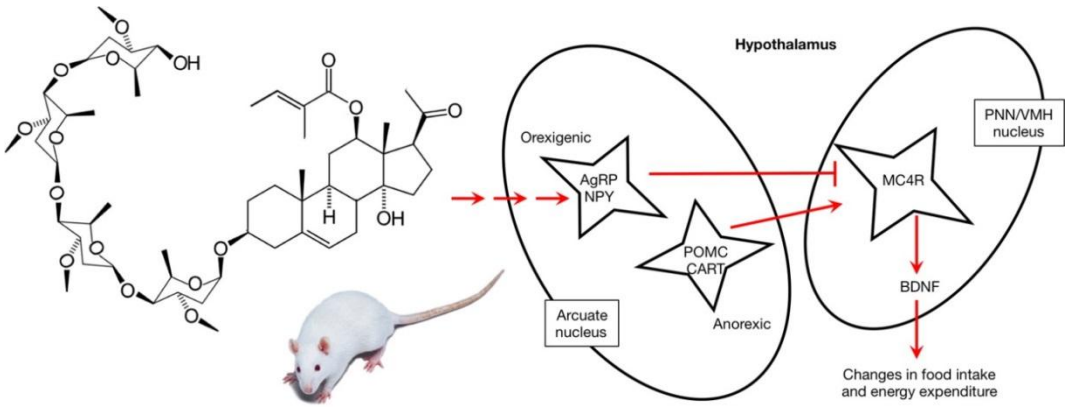


Figure 2.7 Proposed mechanism of action of pregnane glycosides on inhibition of food intake (Komarnytsky et al., 2013).

2.4.2.7 Safety evaluation of C. fimbriata

In terms of safety, initially there was an abstract in 2006 and a book chapter in 2007 that briefly reported on an acute oral toxicity and 90-day oral toxicity of *C. fimbriata* in Wistar rats (Venkatesh, 2007). Both studies were reported to not be Good Laboratory Practice (GLP) compliant (Odendaal et al., 2013b). The acute oral toxicity study, performed at St John's Medical College (Bangalore, Karnataka, India), identified no deaths up to and at the highest dosage administered (5 g/kg BW). A non-significant change was reported for food intake, body weight, haematology, or macroscopic histopathology in male and female rats receiving a single dose of *C. fimbriata* at 5 g/kg BW.

The 90-day oral toxicity study, conducted at Bombay College of Pharmacy (Mumbai, Maharastra, India), comprising of 3 doses of *C. fimbriata* (90, 270, and 900 mg/kg BW/d) reported no treatment-related haematological or clinical chemistry abnormalities. Four deaths occurred during that trial (one male in each of the 270, 900, and satellite 900 mg/kg BW/d groups and one female in the 900 mg/kg BW/d group) and were preceded by dramatic weight loss and weakness. Necropsy of the expired rodents did not exhibit any histopathological abnormalities or tissue damage, and the cause of death for these four rodents was not established. Male rats in the 270 and both the 900 mg/kg BW/d groups further exhibited sparse hair and subcutaneous fat loss toward the completion of the trial; shedding steadily dropped during the recovery time. All *C. fimbriata* treated rodents had organ weights and organ-to-body weight ratios comparable to those of the control group, and no treatment related morphological or histopathological changes were discovered upon histopathological analyses. These initial preclinical non-GLP trials give somewhat of an insight into the safety of *C. fimbriata* in Wistar rats.

Odendaal et al (2013) assessed the genotoxicity of *C. fimbriata* in an Ames and chromosomal aberration test and evaluated the botanical extract's toxicological potential in two GLP compliant rodent toxicity studies, specifically, a six month repeated dose chronic oral toxicity study and a developmental toxicity study in Sprague-Dawley rats (Odendaal et al., 2013b). No indication of in vitro mutagenicity or clastogenicity of *C. fimbriata* was identified in the Ames test at concentrations up to 5000 mg of extract/plate or in the in vitro chromosomal aberration assay at concentrations up to 5000 mg of extract/mL, respectively. Both in vivo assessments showed *C. fimbriata* to be nongenotoxic up to the highest concentrations tested in the presence and absence of metabolic activation (Odendaal et al., 2013b). No targeted tissue or organs were identified, and microscopic examination of the upper and lower gastrointestinal tract organs and tissue did not indicate any evidence of treatment related abnormalities. In the 90-day oral toxicity study, where there were four unexplained deaths as well as hair loss and loose skin in male rodents near the completion of the study, none of these events arose in the chronic oral toxicity study. Therefore, prolonged oral administration of *C. fimbriata* to Sprague-Dawley rats resulted in no toxicological outcomes. Furthermore, no maternal or embryonic-fetal development abnormalities appeared in the prenatal developmental toxicity test at levels up to 1000 mg/kg BW/d in Sprague- Dawley rats. The study demonstrated that there was no evidence of maternal, pregnancy, litter, or fetal toxicity at and up to the highest dose tested. Thereby, the results of this toxicological assessment revealed that *C. fimbriata* is not associated with any toxicity or adverse events (Odendaal et al., 2013b).

A recent study conducted by Rajendran et al., (2008) investigated the nootropic activity of *C. fimbriata* extract in mice. An acute toxicity study on male albino mice was performed as per

OECD 2001 guidelines. The mice were administered different doses of *C. fimbriata* and it was found to be non-toxic up to the dose of 2000 mg/kg BW (Rajendran et al., 2008).

2.4.2.8 Clinical trials investigating the effect of C. fimbriata on metabolic abnormalities

While the effect of *C. fimbriata* on appetite suppression has not been elucidated, it has been demonstrated that the perennial herb is capable of inhibiting adipocyte maturation. A study on 3T3-L1 pre-adipocyte cell line samples conducted by Akbarsha et al., (2010) clarified the mechanism of action of the pregnane glycosides. The study showed that pregnane glycosides have anti-adipogenic properties through the inhibition of pre-adipocyte cell division in the early phase of adipogenesis by either the down-regulation of cyclin-dependent kinase (CDK) or inhibition of import cyclin D1-CDK/6 complex into the nucleus, resulting in G1 arrest. Thus, *C. fimbriata* has the potential to block hyperplastic obesity (Akbarsha et al., 2010). Adipocyte proliferation and differentiation in adipose tissue has been inhibited by pregnane glycosides in other studies (De Leo et al., 2005, Plaza et al., 2005, Cioffi et al., 2006).

A rat study investigating the anti-obesogenic and anti-atherosclerotic properties of *C. fimbriata* showed that following *C. fimbriata* administration there was a significant reduction in food intake and prevented weight gain in body weight, liver weight and fat pad mass (Kamalakkannan et al., 2010). Hyperleptinaemia and leptin resistance were eliminated by the plant extract and the accumulation of lipids in the intima of the thoracic aorta was inhibited, which may be mediated by the improvement in plasma lipid profile (Figure 2.8a,b) (Kamalakkannan et al., 2010).

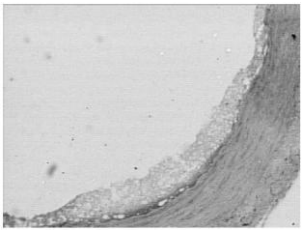


Figure 2.8a Atherosclerosis in aorta of cafetaria fed rat (108x81mm)

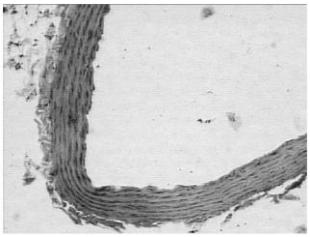


Figure 2.8b Aorta section of rat fed Cafeteria diet plus *C. fimbriata* extract (108x81 mm)

The study by Ambadasu et al (2013) also showed that administration of *C. fimbriata* for 50 days has anti-obesogenic effects. Ambadasu et al (2013) showed that there was a significant reduction in food intake, body weight and blood lipid profile in obese rats treated with *C. fimbriata* (Ambadasu et al., 2013a). Another study by Ambadasu et al (2013) also demonstrated that 50 days of *C. fimbriata* administration resulted in appetite suppressing, hypolipidemic and anti-obesogenic effects in rats fed a hypercaloric diet (Ambadasu et al., 2013b).

An animal study conducted by Sudhakara et al (2013) also demonstrated that administration of *C. fimbriata* for 90 days prevented weight gain and hyperleptinemia (Sudhakara et al., 2014). This study also found that *C. fimbriata* administration prevented hypertriglyceridemia, hyperglycemia and partially prevented hyperinsulinemia, as well as causing a reduction in oxidative stress and plasma glucose levels (Sudhakara et al., 2014). The reduction of leptin and insulin levels in *C. fimbriata* treated groups may be attributed to a reduction in fat accumulation in the adipose tissue and to weight loss. It is therefore suggested that *C. fimbriata* supplementation may be a useful therapeutic target to curtail insulin resistance, oxidative stress and obesity in high fat fed Wistar rats.

The hypolipidemic activity of *C. fimbriata* was investigated in another rodent study conducted by Somnath et al (2012). This study found that treatment with *C. fimbriata* after 15 days significantly reduced total cholesterol and triglycerides in triton induced hyperlipidemic Wistar rats. Furthermore, the atherogenic index was found to be significantly reduced in the *C. fimbriata* treated groups (Somnath et al., 2012). The anti-hyperglycemic and lipid lowering activity of *C. fimbriata* was also investigated in the study by Jagtap et al (2013). This study found a reduction in serum glucose, total cholesterol, triglycerides and LDL cholesterol in dexamethasone induced diabetic Sprague-Dawley rats treated with *C. fimbriata* after 11 days of administration (Jagtap et al., 2013). A more recent study conducted by Latha et al (2014) investigated the hepatoprotective and anti-diabetic effects of *C. fimbriata* in streptatozocin induced diabetic rats. Latha et al (2014) also observed a significant reduction in blood glucose levels in *C. fimbriata* treated rats. Furthermore, histological examination of the liver and kidney in *C. fimbriata* treated rats confirmed a protective action of *C. fimbriata* as there was a significant recovery of liver and kidney destruction observed. Therefore *C. fimbriata* may be seen as a therapeutic target for diabetes and its related complications (Latha et al., 2014).

The mechanism of hypoglycemic activity of *C. fimbriata* is yet to be determined. However, as *C. fimbriata* contains many chemical constituents including pregnane glycosides, flavonoids, megastigmane glycosides, bitter principles and saponins, it may be suggested that these chemical constituents could be responsible for the hypoglycemic activity. A possible mechanism by which this botanical decreases blood glucose is by the potentiation of insulin effect, through either stimulating pancreatic secretion of insulin from β-cells of islets of Langerhans or via enhancing peripheral glucose uptake. This proposed anti-diabetic mechanism of *C. fimbriata* is similar to that of *Caralluma edulis* (Wadood et al., 1989). The

histopathological changes seen in the diabetic group were restored in the *C. fimbriata* treated rats. Hence, further research into the mechanism of hypoglycemic effects of *C. fimbriata* can be undertaken through oral glucose tolerance tests, insulin sensitivity tests, measurement of urinary output and analysis of cytokines (diabetic markers) for instance.

A study conducted by Saivasanthi et al (2011) evaluated the anti-inflammatory effects of *C. fimbriata* in Wistar rats. Anti-inflammatory activity of *C. fimbriata* was evaluated by using carrageenan induced paw oedema model of rat (Saivasanthi et al., 2011). The progression of oedema in the paw of the rat after injection of carrageenan is a biphasic event (Vinegar et al., 1969). The initial phase of the oedema has been attributed to the release of histamine and serotonin, the oedema maintained during the plateau phase to kinin like substances and the second accelerating phase of swelling to the release of prostaglandin like substances (Asongalem et al., 2004). Therefore, the inhibition of oedema following *C. fimbriata* administration may be attributed to the ability of *C. fimbriata* to inhibit various chemical mediators of inflammation, such as histamine and 5-HT during the initial phase. It is also postulated that the anti-inflammatory activity of *C. fimbriata* could be achieved through inhibition of cyclooxygenase, which is involved in the synthesis of inflammatory prostaglandins as it has been demonstrated that *C. fimbriata* contains flavonoids which are known to possess anti-inflammatory properties (Saivasanthi et al., 2011). Further research into the underlying mechanisms involved in the anti-inflammatory effects of *C. fimbriata* is needed. In addition, future directions may be focused on the effect of *C. fimbriata* on metabolic syndrome associated inflammation through analysis of anti-inflammatory and pro-inflammatory cytokines.

There is limited research into the effect of *C. fimbriata* on humans and therefore it has been of interest to verify these therapeutic claims observed in rodents through human controlled

clinical studies. A preliminary clinical trial conducted by Kurpad et al., (unpublished) at the St John's Medical College in Bangalore, India showed significant weight reductions in overweight subjects with *C. fimbriata* supplementation (Preliminary data). Another preliminary clinical trial conducted by Lawrence et al., (unpublished) in Los Angeles, California, USA also showed a significant reduction in body weight with *C. fimbriata* ingestion (Lawrence and Choudhary, 2004).

A human trial evaluating the appetite suppressing effects of *C. fimbriata* in Indian adults found that the botanical extract (1g/day) appears to suppress appetite and reduce waist circumference in overweight individuals (n = 50) with a BMI greater than 25 kg/m^2 over a two month period compared to the placebo group (Kuriyan et al., 2007). However, the experimental group was not significantly different from the placebo group in body weight, BMI, hip circumference and percentage body fat. The adverse effects experienced by 24 % of subjects in the experimental group were minor and restricted to initial mild symptoms of the gastro-intestinal tract which subsided within a week; these included abdominal distension, constipation, flatulence and gastritis.

Kuriyan et al., (2007) also found that hunger levels of participants reduced by 20 % following the administration period, which may account for an 8 % decline in energy intake of the experimental group. However, this could be influenced by under estimation of food intake and/or individual variation. The appetite suppressing effect caused a decrease in energy and fat intake and also a decline in the consumption of less desirable food (Kuriyan et al., 2007). Furthermore, there was no significant change in blood lipid values in the experimental group. As there is only one published human clinical trial that has investigated the effect of *C. fimbriata* extract on appetite and obesity, there is a need to clarify and further investigate the

potential beneficial effects of this popular weight loss aid in the treatment of metabolic syndrome.

2.4.3 Citrus sinensis

2.4.3.1 History, distribution and morphology of C. sinensis

The exact origin of this commonly named red orange is not known, however it possibly originated from China or southern Mediterranean regions. Citrus fruit may have been introduced in Sicily by Arab traders during the 7th century and cultivated for decoration until the 16th century. The red orange was first described as a strongly pigmented type of orange fruit in Sicily in the 17th century opera *Hesperidies* (1646). The Moro variety is thought to have originated in the early 19th century in the citrus-growing area near Lentini (in the Province of Siracusa in Sicily) as a bud mutation of the red orange native to Spain *"Sanguinello Moscato".* The Sanguinello variety is also present in Sicily described as a full-blood orange with close characteristics of the Moro orange. The Moro orange is the most colourful of the red orange varieties. It is referred to as the "deep blood orange" as it has deep red flesh which ranges from orange-veined with ruby colouration, to vermilion, to vivid crimson and nearly to black and a rind that has a bright red blush (Figure 2.9). This type of fruit has a distinct sweet flavour with a hint of raspberry and the intense red to purple pigment makes the fruit more attractive.

Figure 2.9 The sweet Moro orange with blood orange flesh

2.4.3.2 Chemical constituents of C. sinensis

The red colouration of the red orange is due to the presence of water-soluble anthocyanin pigments, the largest pigment class of flavonoids. This class of pigmented molecules have many therapeutic properties beneficial for human health (Cotroneo, 2006). Anthocyanins are natural occurring polyphenolic compounds that are widely distributed in plant foods such as crops, fruits, beans, red wine, pigmented cereals and vegetables (Tsuda et al., 2004). Studies have shown a link between moderate consumption of anthocyanins through red wine and a lower risk of metabolic syndrome and CHD (Renaud and de Lorgeril, 1992). Generally, under acidic conditions, anthocyanin pigments are stable, however under neutral conditions they are unstable and rapidly break down (Brouillard and Cheminat, 1988).

Orange trees are grown in specific environmental and climatic conditions therefore increasing the level of pigmentation and bioactive compounds within the fruit. The phytochemical substances identified in *C. sinensis* include: sugars i.e. sucrose, fructose and glucose; organic acids (chiefly citric, isocitric and malic acids); carotenoids for instance carotenes and xanthophylls; vitamins such as vitamin A, B_1, B_6, B_3, and C; flavour compounds such as alcohols, esters, lactones, ketones, volatile hydrocarbons and polyphenols including hydroxycinnamic acids and flavonoids (Titta et al., 2010). The content of these substances

however differs significantly between cultivars, as cultivation is affected by environmental influences including soil, climate and agricultural procedures and fruit maturation (Rapisarda, 2001).

There are three main cultivated varieties of *C. sinensis* which include: *Tarocco, Moro* and *Sanguinello*. These varieties are different from the common orange due to the presence in the flesh or the rind of the red pigments identified in the anthocyanin class (Rapisarda, 2001). Furthermore, another feature of the blood orange is the high content of vitamin C (4.3-4.5 % p/p) (Rapisarda, 1996), hydroxycinnamic acids (0.8-1.0 % p/p) (Rapisarda et al., 1998) and flavonoids (2-2.2 % p/p) (Postorino, 1999). The blood oranges also have a high concentration of anthocyanins (0.8-0.9 % p/p).

2.4.3.3 Clinical trials investigating the effect of C. sinensis on metabolic abnormalities

Anthocyanins are considered to be the largest group of water soluble pigments of the plant kingdom (Tsuda, 2008). The most predominate anthocyanin present in blood orange is the cyaniding-3-glucoside (C3G) which has been shown to ameliorate insulin resistance (Tsuda et al., 2003), suppress the development of obesity, reduce fat accumulation (Tsuda et al., 2003, Jayaprakasam et al., 2006) and normalize hypertrophy of the adipocyte in epididymal adipose tissue (Tsuda et al., 2003, Titta et al., 2010) when administrated to high fat fed mice as shown in Figure 2.10a,b (Titta et al., 2010). These results indicate that anthocyanins are capable of regulating obesity and insulin sensitivity and therefore reducing the risk of metabolic syndrome.

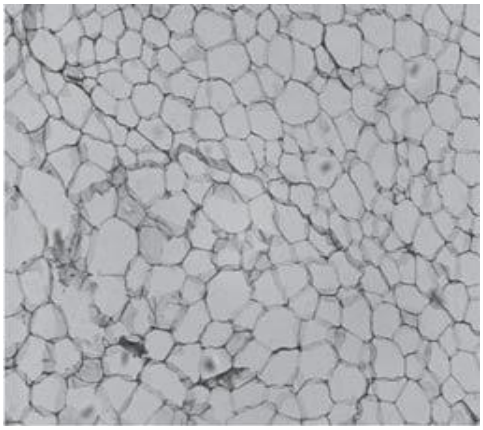


Figure 2.10a Epididymal adipose tissue of a mouse fed high fat diet

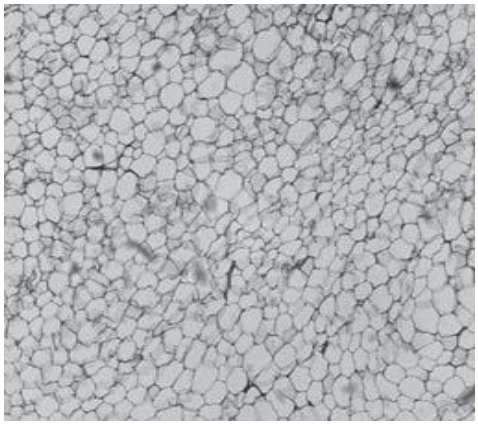


Figure 2.10b Epididymal adipose tissue of a mouse fed high fat diet + Moro

These water soluble pigments are also known to act as contributors to antioxidant activity and anti-free radical activity (Lo scalzo, 2004). At the molecular level, anthocyanins have been reported to act as antioxidants playing a protective role from lipid, protein and DNA damage (Acquaviva et al., 2003). It is hypothesized that anthocyanins may potentially reduce oxidative stress through activating certain detoxification enzymes including quinine oxidoreductase, glutathione S-transferase, glutathione peroxidise and glutathione reductase (Shih et al., 2007).

Studies have also found that at the cellular level anthocyanins block 12-O-tetradecanoylphorbol-13-acetate induced inflammation by inhibiting the mitogen-activated protein kinase pathway in a mouse epidermal cell line, thereby inhibiting tumorigenesis (Hou et al., 2004). These results suggest that anthocyanins exert an antiinflammatory action through normalizing adipocytokine expression and exert antitumor activities. Anthocyanins have also been shown to express a protective role against TNF-α-induced insulin resistance and H_2O_2 when administered to 3T3-L1 adipocytes through the inhibition of c-Jun NH2-terminal kinase activation (Guo et al., 2008). Moreover, it has been demonstrated that

anthocyanins enhance adipocytokine secretion namely leptin and adiponectin, the expression of peroxisome proliferator activated receptor (PPAR) γ and adipocyte specific genes in isolated rat adipocytes (Tsuda et al., 2004).

Another study conducted by Tsuda et al., (2006), looking at the gene expression profile in human adipocytes treated with anthocyanins found significant changes in the expression of adipocytokines including the up-regulation of adiponectin and the down regulation of PAI-1 and IL-6, which are associated with metabolic syndrome. Furthermore lipid metabolism related genes including uncoupling protein 2, acylCoA oxidase 1 and perilipin were up-regulated following anthocyanin treatment. The up-regulation of these genes may limit excess lipid accumulation in adipocytes (Tsuda et al., 2006). These studies imply that anthocyanins are capable of regulating adipocytokine gene expression thereby ameliorating adipocyte function which is associated with the major metabolic syndrome components including obesity and insulin resistance.

Tsuda et al., (2005) also found that lipolytic activity was significantly enhanced in C3G treated rat adipocytes. The elevation in lipolytic activity following C3G treatment could be attributed to the elevation in the expression of hormone sensitive lipase (HSL) induced by anthocyanins (Tsuda et al., 2005). Tsuda et al., (2005) also demonstrated that following the treatment with C3G this resulted in a significant release in glycerol, however the release of free fatty acids (FFA) did not change. Studies have shown that leptin administration increases the release of glycerol from adipocytes (Siegrist-Kaiser et al., 1997, Wang et al., 1999) and leptin stimulation is not accompanied by significant amounts of FFA release (Wang et al., 1999). As mentioned earlier, previous studies have shown that anthocyanins enhance the expression of adiponectin and leptin secretions in adipocytes (Tsuda et al., 2004). These findings may be associated with the elevation in lipolytic activity such that the

anthocyanin treated adipocytes results in the stimulation of leptin expression and secretion, thereby increasing the release of glycerol without enhancing FFA release (Tsuda et al., 2005). By suppressing FFA release from the adipocytes this is beneficial for improving insulin sensitivity in peripheral tissues. It is also hypothesised that leptin enhances fatty acid oxidation and is involved in regulating intracellular lipid levels, and anthocyanins can modulate this regulation through the expression of leptin and/or HSL (Tsuda et al., 2005).

Buscemi et al., (2012) found that after 7-days of red orange juice consumption (500 mL red orange juice/ day) this resulted in the amelioration of endothelial function and reduced inflammation in non-diabetic subjects at an increased risk of cardiovascular disease. Endothelial function is measured via flow-mediated dilation in the brachial artery. Flow-mediated dilation is a strong predictor of cardiovascular events (Yeboah et al., 2007). Buscemi et al., (2012) found that following administration of red orange juice, flow-mediated dilation significantly improved. Furthermore, blood concentrations of inflammatory markers including CRP, IL-6 and TNF-α significantly reduced after one week of red orange juice consumption. Therefore the observed reduction in inflammatory markers could have beneficial effects on atherosclerotic progression (Buscemi et al., 2012). Although this study has shown favourable effects of red orange juice consumption on inflammation and endothelial function in the short term, there is a need for intervention studies of a longer duration to test the effects of this orange on metabolic and cardiovascular endpoints.

Hypertension is a very common progressive condition that is associated with an increased risk of many chronic diseases including CVD, stroke and diabetes. The inhibition of ACE shows to be a promising way of controlling the over expression of RAAS. Studies have shown that anthocyanins show ACE inhibition in vitro. Administration of plant-derived purple corn, purple sweet potato and red radish (rich in anthocyanins) has been shown to

improve systolic and diastolic blood pressure in hypertensive rats (Shindo et al., 2007). The reported ACE inhibitory activity of anthocyanins in vitro may be attributed to the metal chelating ability of flavonoids with hydroxyl groups at 3, 5, 7 and 3', 4' positions (Kwon et al., 2010, Persson et al., 2009). The planer structure of the anthocyanin molecules also indicated to be imperative in metallopeptidase inhibition (Ojeda et al., 2010). Nonetheless, a strong correlation between ACE inhibition in vitro and animal model systems is yet to be investigated. Furthermore, enzyme kinetic studies have focused on establishing the type of enzyme inhibition of flavonoids. The majority of flavonoids were found to be competitive type inhibitors meaning that they can compete with the substrate in binding to the active site of the enzyme (Balasuriya and Rupasinghe, 2012).

A study conducted by Bonina et al., (2002) demonstrated that following two months administration of red orange complex (*C. sinensis* var Moro, Tarocco & Sanguinello), there was an improvement in blood levels of thiol groups on proteins in participants with diabetes, in addition to a marked reduction in serum free radical levels in subjects with high blood oxidative stress status. Therefore this study identified the potential therapeutic benefits of red orange complex supplementation as a preventative tool for diabetes complications associated with uncontrolled lipid oxidation (Bonina et al., 2002). Another study conducted by Bonina et al., (2005) found that after two months supplementation with the red orange complex (*C. sinensis* var Moro, Tarocco & Sanguinello) there was a reduction in biomarkers of oxidative stress in professional handball players. A sex- and age-matched control group of sedentary, healthy individuals (17 males, aged 19-30 years) were used as controls. Serum lipid peroxidation was determined by lipid hydroperoxides, which was found to be significantly decreased in the red orange treated group, suggestive of a normal oxidative stress status. Furthermore, serum total antioxidant status and serum level of thiol groups were completely

restored in the handball players following supplementation. Circulating malondialdehyde levels, a marker of oxidative damage, was markedly higher at the beginning of the study in the handball players compared to the control group. However, by the end of the intervention, circulating malondialdehyde levels returned towards control values (Bonina et al., 2005). In addition, Bonina et al., (2005) also reported that administration of red orange extract was well tolerated by all subjects, with no unpleasant side effects noted.

A human clinical trial conducted by Dallas et al., (2008) demonstrated that supplementation with SINETROL (combination of red orange, grapefruit & orange) may prevent obesity through a reduction in BMI. There was a significant reduction in percentage body fat and body weight in the SINETROL treated group compared to placebo following 12 weeks of supplementation. The results of this study suggest that SINETROL has a strong lipolytic effect, which is mediated by cAMP-phosphodiesterase inhibition (Dallas et al., 2008). Following on from this study, Dallas et al (2014) then investigated the efficacy and safety effects of Sinetrol-XPur, containing citrus Polyphenolic Extract of Red Orange, Grapefruit, and Orange on metabolic and anthropometric parameters, inflammation, glycemia and oxidative status in overweight subjects. The clinical trial was conducted over a 12 week period, where participant's diet and exercise was controlled in addition to supplementation of the Sinetrol-XPur. The major findings of this study were a significant reduction in waist and hip circumferences and total abdominal adiposity was also reduced (Dallas et al., 2014). Inflammatory markers including plasma CRP and fibrinogen were significantly reduced and the oxidative status of participants improved through the reduction of malondialdehyde (-14 %) and a rise in superoxide dismutase activity and an increase in plasma glutathione levels (~17 % and ~5 % respectively) (Dallas et al., 2014).

There is a growing body of evidence suggestive of beneficial metabolic effects and cardio-protective properties of red orange varieties rich in anthocyanins. However, the effectiveness of the Moro orange variety of *C. sinensis* on all the major risk factors of metabolic syndrome and the risk of atherosclerosis in the clinical setting is limited. Therefore, there is a need to investigate the efficacy of Moro orange administration on the risk factors of metabolic syndrome in overweight and obese adults.

2.4.4 Conclusions

The increasing prevalence of obesity, metabolic syndrome and associated manifestations including atherosclerosis, and an increase in oxidative stress, adhesion molecules, adipocytokines and inflammatory cytokines has sparked the search for preventative and therapeutic interventions as a means for combating this significant and important public health issue. In view of the potentially beneficial effects of *C. fimbriata* and *C. sinensis* extracts as reviewed in this chapter, the joint administration of these botanicals appears to be a promising therapeutic strategy in the fight to treat the major components of metabolic syndrome. To date, there are no studies that have evaluated the efficacy of *C. fimbriata* extract plus *C. sinensis* extract for the treatment of metabolic syndrome, despite the increasing market and prescriptions for plant-based body weight control therapies.

SECTION IV: AIMS AND HYPOTHESES

2.5.1 Overarching aim:

The overall aim of this thesis is to determine the effects of *C. fimbriata* extract on the risk factors of metabolic and cardiovascular disorders in overweight and obese conditions compared to placebo.

2.5.2 Study 1:

The first aim of this thesis was to determine whether *C. fimbriata* extract, in addition to a hypocalorie diet and regular physical activity, can attenuate metabolic disturbances including central obesity, elevated BP, dyslipidemia and elevated blood glucose levels in overweight and obese Australian adults compared to placebo.

The specific hypotheses tested were that:

1. Following 12 weeks administration of *C. fimbriata* extract, there would be a reduction in waist circumference, lower blood pressure and fasting blood glucose levels as well as an improvement in blood lipid profile compared to placebo.

2. Following 12 weeks administration of *C. fimbriata* extract, there would be an increase in satiety, as well as a decrease in energy intake (kJ) compared to placebo.

3. Following 12 weeks administration of *C. fimbriata* extract, there would be a reduction in the adipocytokine leptin compared to placebo.

2.5.3 Study 2:

The second study aimed to determine whether *C. fimbriata* attenuates the metabolic changes produced in an obesity-inducing rat model compared to placebo.

The specific hypotheses tested were that:

1. Following 8 weeks administration of *C. fimbriata* extract, there would be a reduction in abdominal adiposity (fat pads, waist circumference & % body fat), lower blood pressure, and an improvement in insulin sensitivity, glucose tolerance and a reduction in plasma cholesterol and triglyceride levels compared to placebo.
2. Following 8 weeks administration of *C. fimbriata* extract, there would be a reduction in hepatic lipid content, hepatic lipid droplets and liver weight compared to placebo.
3. Following 8 weeks administration of *C. fimbriata* extract, there would be an increase in satiety, as well as a decrease in feed consumption compared to placebo.

2.5.4 Study 3:

The third study aimed to explore the effect of *C. fimbriata* in combination with *C. sinensis* on the risk factors of metabolic syndrome and cardiovascular risk factors compared to placebo in a randomised controlled clinical trial.

The specific hypotheses tested were that:

1. Following 12 weeks administration of *C. fimbriata* extract plus *C. sinensis*, there would be a reduction in percentage body fat, a decrease in body circumferences specifically waist circumference and an improvement in obesity adipocytokines compared to placebo.

2. Following 12 weeks administration of *C. fimbriata* extract plus *C. sinensis*, there would be an improvement in blood pressure, fasting blood glucose levels, plasma insulin, HOMA as well as an improvement in blood lipid profile compared to placebo.

3. Following 12 weeks administration of *C. fimbriata* extract plus *C. sinensis*, there would be an increase in satiety, as well as a decrease in energy intake (kJ) and less desirable food choices and an increase in desirable food choices compared to placebo.

4. Following 12 weeks administration of *C. fimbriata* extract plus *C. sinensis*, there would be a reduction in the atherosclerotic biomarkers as well as the reduction in the obesity adipocytokines compared to placebo.

CHAPTER 3: GENERAL MATERIALS AND METHODS

3.1 Experimental outline of human studies, participant recruitment and randomisation

Both human studies were randomized, double-blinded, placebo controlled trials, involving adult volunteers residing in Melbourne. The trials were conducted at Victoria University, Nutrition Clinic, Melbourne, Australia. Potential volunteers were recruited through the general public and staff members at Victoria University. Human volunteers with a BMI greater than 25 kg/m^2 or a waist circumference > 94 cm (male), > 80 cm (female) were randomly assigned in the treatment or placebo group.

For randomisation, participants were coded and allocated into groups based on their physical characteristics including age, body weight, height, BMI, waist and hip circumference and WHR. The method used to ensure allocation concealment was sequentially numbered containers. The containers were equal in weight, similar in appearance and tamper-proof. The principal investigator implemented the allocation sequence and assigned the participants into their groups. The capsules were opaque and indistinguishable in appearance, size, texture and smell. The taste of the capsules was identical provided that they were swallowed whole as instructed. Staff and participants involved in the intervention process of the trial were blinded to group assignment. The randomisation code was broken only after data collection and statistical analysis was completed. Patient and staff blinding to treatment was monitored throughout the study. This was evaluated by asking participants whether they thought they were in the treatment or placebo group and staff were also asked if they knew whether their participant was in the treatment or placebo group. The inclusion and exclusion criteria for the study are presented in Table 3.1.

Table 3.1: Eligibility criteria for entry into the study

Inclusion Criteria	Exclusion criteria
Male or female	Cigarette smoker
Aged between 20 – 60 years	Heart, liver, kidney disease
BMI >25 kg/m^2 or	Pregnant, intending to become pregnant, women lactating
Waist circumference >94 cm (male), >80 cm (female)	Type 1 diabetes
Residing in Melbourne	Currently taking medications that influence metabolism or affect appetite

The pilot study (Chapter 4) was approved by the Human Research Ethics Committee of Victoria University, Australia (HRETH 10/22) and registered by Australian New Zealand Clinical Trials Registry (ANZCTR). The major human study (Chapter 6) was also approved by the Human Research Ethics Committee of Victoria University, Australia (HRETH 12/264) and registered by ANZCTR. At the beginning of the study all eligible volunteers were informed about the details of the study including that they would be randomly assigned into a treatment or placebo group. Formal consent was obtained from all participants.

3.1.1 Administration of botanical extracts

For the pilot study the control capsule was a 500 mg capsule containing 100 % maltodextrin. The *C. fimbriata* extract (min 25 % pregnane glycosides and min 10 % saponin glycosides) and placebo capsules were provided in a 2-piece hard shell capsule form and delivered in blindly labelled sealed bags as 500 mg capsules twice daily (1g/day) before meals for 12 weeks. This dosage was determined based on the previous study by Kuriyan et al (2007).

For the major human trial, there were four groups. Group 2 was given a daily dose of *C. fimbriata* (1g of *C. fimbriata* extract per day: min 25 % pregnane glycosides and min 10 % saponin glycosides), group 3 was given a daily dose of *C. sinensis* (500 mg of *C. sinensis*: min anthocyanins 0.8-0.9 %, flavonoids 2-2.2 %, hydroxycinnamic acid 0.8-1.0 % and ascorbic acid 4.3-4.5 %), group 1 was given a daily dose of a combination of *C. fimbriata* and *C. sinensis* and group 4 was given a placebo (100 % maltodextrin) in two equally divided doses 30 minutes before two meals each day for 12 weeks. The capsules were provided in a 2-piece hard shell capsule form and delivered in blindly labelled sealed bags. The dosage of *C. fimbriata* was determined based on the our previous study (Astell et al., 2013a). The dosage of *C. sinensis* was determined based on the previous pilot study conducted at Victoria University (Ethics no. HRETH11/43). Both capsules were supplied by Gencor Pacific, Hong Kong. The ingestion of the capsules was monitored during the nutrition consultations and a capsule calendar was also administered at the beginning of the trial and submitted at the completion of the 12 weeks. Volunteers were asked to record their capsule consumption daily and noted down if they missed taking any capsules, as well as any adverse effects experienced throughout the 12 weeks. The compliance of capsule ingestion was measured as a percentage of total capsules consumed.

3.1.2 Experimental design of pilot study and major human trial

Participants were asked to attend the Victoria University Nutrition Clinic, Melbourne, Australia for nutrition consultations on a weekly basis for the pilot study and only fortnightly for the major human trial. During the intervention period participants were asked to control their dietary intake and exercise according to the advice provided by the student researcher. All groups received consistent dietary advice provided by the student researcher (qualified

nutritional therapist) during each consultation. In order to obtain optimal compliance among participants, it was deemed helpful to provide and control participant's dietary intake and physical activity. Participants were asked to maintain their usual participation in physical activities during the trial period. For the major human trial physical activity was monitored by providing volunteers with a physical activity calendar and a physical activity questionnaire was provided at baseline and post intervention. Dietary intake was monitored through 3-day food diaries and submitted monthly in both studies and only for the major human trial a food frequency questionnaire was completed at baseline and post intervention.

The participants followed a hypocaloric diet (deficit of approximately 500kcal/day of estimated energy requirements) based on the Adult Weight Management Evidence-Based Nutrition Practice Guidelines (Academy of Nutrition and Dietetics, 2010). In addition, the nutrition recommendations provided were in accordance with the Dietary Guidelines for Australian Adults (Australian Government, 2005) and the Clinical Practice Guidelines for the Management of Overweight and Obesity in Adults (NHMRC., 2003). Anthropometric measurements and BP were taken during each nutrition consultation. Appetite sensations, body composition parameters and biochemical analyses were measured at baseline and post intervention.

3.2 Outcome measures of human studies

3.2.1 Anthropometric measurements

Height was measured after the removal of shoes using a stadiometer to the nearest millimetre. Body weight was taken using digital scales when heavy clothing was removed. BMI was

calculated using the following formula: BMI = weight (kg)/height $(m)^2$. Waist circumference was measured to the nearest 0.1 cm at the midway point between the lowest costal border and the iliac crest in a horizontal plane. Hip circumference was measured in a horizontal plane at the maximum posterior protuberance of the buttocks. WHR was calculated using the following formula: WHR = Waist circumference (cm)/hip circumference (cm).

The three dimensional laser scanner employed for the major human study to obtain 3D digital images of the human form was TC^2. Percentage body fat was measured with the 3D laser scanner. Participants wore fitted, light-coloured undergarments with hair tied back. Breathing remained normal throughout the scan and feet were placed on the foot pads, slightly apart. Participants gripped onto the handles with arms straightened, slightly away from the body to allow for each body segment to be measured. Participants remained motionless, however in the event of movement, participants were rescanned. Data acquisition and processing of the 3D body image was completed within a minute.

3.2.2 Blood pressure

An appropriately sized blood pressure cuff was used to measure blood pressure. Blood pressure was measured in a seated position using an automated digital BP monitor at each nutrition consultation (UA- 767 Plus, A & D Medical), where the lower edge of the cuff of the sphygmomanometer was positioned one inch above the antecubital fossa. The flexed elbow was positioned at the level of the heart. If a participant was feeling anxious, blood pressure was taken later in the consultation when the participant was more relaxed. BP was measured twice with the final BP reading obtained by calculating the mean of the two readings. HR was also displayed on the digital BP monitor screen and recorded.

3.2.4 Dietary and physical activity assessment

Subjects were given detailed instructions on how to measure and record their food and beverage intake before commencing the study. Subjects were advised to record detailed descriptions of foods and beverages such as type, amount, brand, time, location, cooking method and ingredients in recipes. The student researcher checked the completed records for errors and clarifications were made with subjects if required. Subjects were required to complete 3-day food diaries at baseline, midway and post-intervention. From the diet diary records, participant's mean total energy intake (kJ), carbohydrate (g), fat (g), protein (g), alcohol (g), saturated fat (g), sugar (g), fibre (g), and salt (mg) intake were analysed using nutrient analysis software, Food Works Professional (Version 7, Xyris Software, Highgate Hill, Queensland, Australia) (Xyris Software Pty Ltd, 2007).

At baseline and post intervention of the major human trial, dietary information was also collected with use of the Cancer Council of Victoria's Food Frequency Questionnaire (FFQ) known as the Dietary Questionnaire for Epidemiological Studies Version 2 (DQESV2). The self-administered 74-item optical mark readable semi-quantitative FFQ has been previously validated (DQESV2) (Giles GG. and Ireland PD., 1996).

The first page of the FFQ consists of questions about how many pieces of fruit and vegetables are consumed daily, amount and type of bread and milk consumed, the type of fat spread used, the amount of sugar consumed daily and the weekly intake of eggs and type of cheese eaten.

The second page of the questionnaire includes four sets of photos depicting three different serving sizes for potatoes, vegetables, steak and meat/vegetable casserole. For each type of food, photograph A = 25^{th} percentile, photograph B = median, photograph C = 75^{th} percentile of the distribution of serving sizes stated in the study by Hunter et al (Hunter et al., 1988).

There are seven different serving portion sizes for each food class which include: less than A, A, between A and B, B, between B and C, C, and more than C. There is also the "I never ate" option for selecting nil intakes. For pages three and four of the FFQ, there are 74 items with 10 frequency options which include: Never, less than once per month, 1 to 3 times per month, 1 time per week, 2 times per week, 3 to 4 times per week, 5 to 6 times per week, 1 time per day, 2 times per day and 3 or more times per day. The list of foods are categorised into four sections which include: 1) cereal foods, sweets & snacks; 2) Dairy products, meat & fish; 3) Fruit; 4) Vegetables including fresh, frozen and tinned. Also, there are three questions regarding alcohol intake which include: 1) How many times; 2) Total number of glasses per day; 3) Maximum number of glasses per 24 hours.

All questionnaires were collected and checked for errors and missing responses by a member of the clinical trial staff at the time of administration. Completed DQESV2 forms were sent to Cancer Council Victoria for analysis using specialist software based on the Australian NUTTAB 1995 food composition database (Australian Government Publishing Services, Canberra).

Physical activity was assessed in the major human trial using the International Physical Activity Questionnaire (IPAQ) - Long form at baseline and post intervention. The IPAQ has been previously validated (Craig et al., 2003). This version consists of 27 questions assessing the frequency and duration of participation in vigorous-intensity, moderate-intensity, walking activity and time spent sitting. The long, self- administered IPAQ covers four domains which include: 1) Work-related; 2) Transportation; 3) Housework, house maintenance, and caring for family; 4) Recreation, sport, and leisure-time physical activity. The questionnaire also includes questions on time spent sitting as an indicator for sedentary behaviour. For each domain, the number of days per week spent on moderate and vigorous physical activities was

recorded. Walking as part of occupation, transportation and leisure time was also recorded. The IPAQ scoring protocol was used to assess total weekly physical activity.

For the purpose of this study, insufficient physical activity was defined in accordance with Australia's Physical Activity & Sedentary Behaviour Guidelines for Adults. Participants who reported <150 minutes (2 ½ hours) of moderate intensity physical activity or <75 minutes (1 ¼ hours) of vigorous physical activity per week were considered to be insufficiently active. Similarly, participants who participated in >150 minutes (2 ½ hours) of moderate intensity physical activity or >75 minutes (1 ¼ hours) of vigorous physical activity per week were considered to be active.

Throughout the study period, participants were asked to keep a daily record of their physical activity. Participants were provided with a physical activity calendar at baseline and were required to submit their calendar at the completion of the study.

3.2.3 Appetite assessment

The appetite of subjects was assessed at baseline and post intervention using the visual analogue scales method (VAS). Subjects were asked to come to the laboratory after an 8 hour overnight fast. Subjects were asked to consume a standardized meal for dinner the night before the study day and to abstain from alcohol and strenuous physical activity for 24 hours prior to the study-day. The cold buffet-style breakfast was served between 07.30 and 10.30 hours in order to replicate the usual breakfast time of each participant. The cold buffet-type meal was composed of a variety of foods to measure macronutrient preferences and spontaneous intake under conditions reproducing free-living conditions. Portions of each food were larger than the expected intake and the amount of food offered was in excess of

what the subject was expected to consume. The nutrient composition of the cold buffet style breakfast is provided in Table 3.2. A large diversity in protein, lipid, and carbohydrate sources was available in order to facilitate the detection of macronutrient preferences: dairy products (milk, yogurt, cheese and iced coffee), fruit and vegetables (Tomato, apple, banana, orange juice), grain products (breads, cereal), meat (Deli ham slices), fat food products (margarine, peanut butter), refined sugar products (white bread, croissant, muffin, jam, honey), and food with no energy (water).

The subjects had *ad libitum* access to food and were given a maximum of 30 minutes to consume the meal and were instructed to eat until satiety was reached. At baseline (t = -30min), immediately after eating (t = 0min) and then at 30-min intervals (t = 30, 60, 90, 120, 150, 180) for 3 hours, subjects were asked to record their subjective sensations of appetite, including hunger, fullness, nausea, drowsiness, anxiety, desire to eat, and prospective consumption at each time interval. The questions asked were 'How hungry do you feel?' (not hungry – hungry); 'How full do you feel?' (not full – full); "How nauseous do you feel? (not nauseous – nauseous), "How drowsy do you feel?' (not drowsy – drowsy), 'How anxious do you feel?' (Calm – Anxious); 'how strong is your desire to eat?' (weak – strong); 'How much food do you think you could eat?' (none – a large amount). Please note that the mood states were only assessed in the major human trial (Chapter 6).

Appetite sensations were assessed using a validated visual analogue scale (Parker et al., 2004). Each visual analogue scale evaluated a sensation on a 100-mm horizontal line, where 0 represented "sensation is not felt at all" and 100 represented "sensation is felt the greatest." Subjects were familiarized with these scales prior to the commencement of the study. Subjects were asked to place a vertical stroke on the 100-mm line in relation to what they

were feeling at that particular point in time. Quantification of the measurement is done by measuring the distance from the left end of the line to the mark. Appetite sensations were assessed both in a fasted state (before breakfast) and in response to the meal. The appetite ratings at baseline and at week 12 were performed in a controlled environment i.e. same room which was kept quiet, free of odours and sight of food. To determine macronutrient preferences and spontaneous intake from the buffet, food items were weighed before and after the buffet to quantify the intake of each food. Energy intake from the buffet meal [energy consumption (in kJ) was analysed using commercially available software (Food works, version 7, Xyris Software; Highgate Hill, Queensland, Australia).

Table 3.2: The nutrient composition of the test breakfast

Nutrient	Amount
Energy	9599 kJ
Carbohydrates	58 %
Fat	27 %
Protein	15 %

The total amount of food at the test meal was measured by weighing food items separately before and after eating to the nearest 0.1g. Once the energy content of the test meal was obtained, the satiety quotient (SQ) was calculated for the degree of hunger appetite sensation (AS) to evaluate the satiating efficiency of the meal (Green et al., 1997) with the following equation:

$$SQ\ (mm/kJ) = \frac{(\text{Fasting AS} - \text{post-meal AS})}{\text{Energy content of test meal (kJ)}} \times 100$$

Satiety quotient represents the effect of food consumption on sensations of appetite relative to energy intake. That is, the higher the quotient, the greater the satiating effect of the test breakfast. To evaluate the mean postprandial period satiety effect of the test meal on measures of satiety (hunger), all time point quotients were averaged (0 – 180 minutes).

Please note that a modified version of the VAS method described above was used for the pilot study. The modified version of this method is described in chapter 4.

3.3 Blood sample collection and Plasma analysis

3.3.1 Blood sample collection

3.3.1.1 Patient preparation

To ensure the procedure was conducted on the right participant, the participant's name was first matched with the consent form by the student researcher. Following this, an explanation of the procedure in plain language to the participant was undertaken. This gave the participant the opportunity to withdraw consent or ask questions to confirm their understanding. In addition, further details were gathered such as time of last meal, hydration status and if the participant was prone to fainting. The room was warm, had adequate lighting, and equipment was at hand and the participant was comfortably seated or lay down.

3.3.1.2 Performance of venepuncture

Performance of safe and competent venepuncture by the student researcher was in accordance with the guidance of the Centre for Education, Western Health. The instructions for

performing venepuncture are as follows: equipment was first gathered, and the participants name was correctly identified by checking the consent form and if there were any special requirements. The equipment was then prepared and pathology tubes were labelled, followed by thoroughly washing hands. Then, a suitable site for venepuncture was determined and safety glasses were put on and gloves were donned. The tourniquet was applied approximately 10 cm above the selected site and the pad of the index finger was used to palpate the antecubital fossa. The proposed puncture site was then cleaned with an alcohol swab and the area was allowed to dry to minimise stinging at the site and to increase the bactericidal effect. Thereafter, the vein was anchored by pulling the skin in a downwards direction, and the needle or butterfly was inserted with bevel up at an angle of about 15-30 degrees. The needle was then swiftly inserted through the skin onto the lumen of the vein. The BD Vacutainer blood collection system and butterfly system were used for this project. The butterfly system was used whenever difficult venepuncture was encountered.

Approximately 10 mL of blood was collected gently into Ethylenediaminetetraacetic acid (EDTA) tubes. When the last tube of blood was collected, the tourniquet was removed. The needle was then removed from the participant's arm using a swift downwards motion and pressure was applied to the puncture site after applying cotton wool over the site (taped if needed). Tubes were gently mixed by inverting the tube 5-6 times and contaminated materials and equipment were disposed of into designated containers, followed by thoroughly washing hands.

3.3.1.3 Centrifuging of whole blood samples

Following collection, blood samples were immediately centrifuged for 10 minutes at 3000 g at 4 °C. The plasma was carefully aliquotted in cryovials and frozen at -80 °C for further analysis of blood lipid profile, obesity adipocytokines and atherosclerotic markers.

3.3.2 Plasma analysis

3.3.2.1 Blood lipids

Blood lipid profile including total cholesterol, HDL cholesterol, and triglycerides were measured by the student researcher from human plasma collected using commercially available kits using a 96-well plate format. LDL cholesterol was calculated using Friedewald's formula (Friedewald et al., 1972). The protocol supplied with the kits for blood lipid profile analysis was designed for a well maintained automated clinical chemistry analyser. Each 96-well plate was analysed using a microplate spectrophotometer. The protocol supplied was followed for the determination of human plasma lipid levels in study 1 and study 3. The same protocol for plasma cholesterol and triglyceride determination was followed for the rodent plasma with use of rat specific kits.

3.3.2.1.1 Total cholesterol

Total cholesterol was determined enzymatically using the commercially available *Infinity*^{TM} Cholesterol Liquid Stable Reagent supplied by Thermo Fisher Scientific Inc. The cholesterol liquid stable reagent is based on the formation of Alain et al (1974) and the modification of Roeschlau et al (1974) with further improvements to render the reagent stable in solution

(Alain, 1974, Roeschlau, 1974). Cholesterol is measured enzymatically in plasma in a series of coupled reactions that hydrolyze cholesteryl esters and oxidize the 3-OH group of cholesterol. One of the reaction by-products, H_2O_2 is measured quantitatively in a perioxidase catalysed reaction that produces a colour. The colour intensity is proportional to cholesterol concentration. The reaction scheme for the enzymatic measurement of total cholesterol is as follows:

Cholesterol Esterase

1. Cholesteryl Ester + H_2O ----------→ Cholesterol + fatty acid

Cholesterol oxidase

2. Cholesterol + O_2 ----------→ Cholest-4-en-3-one + H_2O_2

Peroxidase

3. $2H_2O_2$ + HBA + 4-AAP ----------→ Quinoneimine Dye + $4H_2O$

Where: HBA = Hydroxbenzoic acid; 4-AAP = 4-aminoantipyrine.

The reagent composition includes: Cholesterol oxidase (>200U/L), cholesterol esterase (>500 U/L), peroxidase (>300 U/L), 4-aminoantipyrine (0.25 mmol/L), HBA (10 mmol/L), buffer (50 mmol/L) and surfactants. The assay procedure involves the following steps presented in Table 3.3.

Table 3.3: System parameters of total cholesterol assay

System Parameters	
Temperature	30-37 °C
Primary wavelength	500 nm
Secondary wavelength	660 nm
Assay type	End Point
Direction	Increase
Sample: Reagent ratio	1:100
Sample volume	3 µL
Reagent volume	300 µL
Incubation time	300 seconds
Reagent blank limits	
Low	0.0 AU
High	0.2 AU
Linearity	0-20 mmol/L (0-774 mg/dL)
Analytical sensitivity	62 ΔmA per mmol/L (1.6 ΔmA per mg/dL)

The concentration of total cholesterol was calculated using the following equation:

$$\text{Cholesterol} = \frac{\text{Absorbance of sample}}{\text{Absorbance of calibrator}} \times \text{Calibrator value}$$

3.3.2.1.2 Triglycerides

Triglycerides were determined enzymatically using the commercially available *Infinity*^{TM} Triglycerides Liquid Stable Reagent supplied by Thermo Fisher Scientific Inc. The

methodology is based on previous work by Wako (Product Data Sheet, Triglycerides – G code No 997-69801, Wako Pure chemical Industries Ltd, Dallas TX) and the modifications by McGowan et al and Fossati et al (McGowan, 1983, Fossati, 1982). Triglycerides are measured enzymatically in plasma using a series of coupled reactions in which triglycerides are hydrolysed by lipase to free fatty acids and glycerol. Glycerol is then phosphorylated by ATP with glycerol kinase to form glycerol-3-phosphate and adenosine diphosphate. Glycerol-3-phosphate is oxidised by dihydroyacetone phosphate by glycerolphosphate oxidase producing hydrogen peroxide. In a Trinder (Trinder, 1969) type colour reaction catalysed by perioxidase, the H_2O_2 reacts with 4-aminoantipyrine and 3,5-dichloro-2-hydrobenzene sulfonate to form a red coloured dye. The absorbance of the dye is proportional to the concentration of triglycerides present in the sample. The reaction scheme for the enzymatic measurement of triglycerides is as follows:

Lipase

1. Triglycerides + H_2O ----------→ Glycerol + Free fatty acids

Glycerol kinase

2. Glycerol + ATP ----------→ Glycerol-3-phosphate + ADP

GPO

3. Glycerol-3-phosphate + O_2 ----------→ DAP + $2H_2O_2$

POD

4. H_2O_2 + 4-AAP + 3,5 DHBS ----------→ Quinoneimine dye + $2H_2O_2$

Where: GPO = Glycerolphosphate oxidase; DAP = dihydroxyacetone phosphate; POD = peroxidase; 4-AAP = 4-aminoantipyrine; 3, 5 DHBS = 3,5-dichloro-2-hydroxybenzene sulfonate.

The reagent composition includes: ATP (2.5 mmol/L), Mg acetate (2.5 mmol/L), 4-aminoantipyrine (0.8 mmol/L), DHBS (1.0 mmol/L), GPO (>3000 U/L), glycerol kinase (>100 U/L), lipoprotein lipase (>2000 U/L), peroxidase (>300 U/L) and buffer (53 mmol/L). The steps of the assay procedure are presented in Table 3.4.

Table 3.4: System parameters of triglycerides assay

System Parameters	
Temperature	30-37 °C
Primary wavelength	500 nm
Secondary wavelength	660 nm
Assay type	End Point
Direction	Increase
Sample: Reagent ratio	1:100
Sample volume	3 µL
Reagent volume	300 µL
Incubation time	300 seconds
Reagent blank limits	
Low	1.0 AU
High	0.2 AU
Linearity	10 mmol/L (885 mg/dL)
Analytical sensitivity	0.158 ΔmA per mmol/L (0.002 ΔA per mg/dL)

The concentration of triglycerides was calculated using the following equation:

$$\text{Triglycerides} = \frac{\text{Absorbance of sample}}{\text{Absorbance of calibrator}} \text{ X Calibrator value}$$

3.3.2.1.3 HDL cholesterol

HDL cholesterol concentrations were measured using the commercially available *Infinity*^{TM} HDL cholesterol automated reagent supplied by Thermo Fisher Scientific Inc. The basic principles of the method involve two reagents:

Step 1: Lipoproteins including LDL, VLDL and chylomicrons (except HDL) are first removed via selective reaction with cholesterol esterase and cholesterol oxidase that is coupled to a non-coloured endpoint via catalase reduction of the peroxidase by-product.

Step 2: Catalase is inhibited and the remaining HDL cholesterol is specifically reacted with cholesterol esterase and cholesterol oxidase. In the presence of peroxidase the peroxide by-product now reacts with 4-aminoantipyrine and HDAOS (N-(2-hydroxy-3-sulfoprophyl)-3,5-dimethoxyaniline) to form a coloured quinone dye which is measured spectrophotometrically at 600 nm (Izawa, 1997).

The composition of reagent 1 includes: buffer, cholesterol esterase (1200 U/L), cholesterol oxidase (500 U/L), catalase (225 000 U/L), ascorbate oxidase (10 000 (U/L), HDAOS and stabilizers. The composition of reagent 2 includes: buffer, peroxidase (2000 U/L), 4-aminoantipyrine (4 mmol/L), surfactants and sodium azide (0.09 %). The automated procedure of HDL cholesterol is presented in Table 3.5.

Table 3.5: System parameters of HDL cholesterol assay

System Parameters	
Temperature	30-37 °C
Secondary wavelength	600 nm
Mode	End Point
Sample volume	4 µL
Reagent volume 1	300 µL
Reagent volume 2	100 µL
Reaction times	5 minutes + 3 minutes
Linearity	0.08-3.88 mmol/L (3-150 mg/dL)

The concentration of HDL cholesterol was calculated using the following equation:

$$\text{HDL cholesterol} = \frac{\text{Absorbance of sample}}{\text{Absorbance of calibrator}} \text{ X Calibrator value}$$

3.3.2.1.4 LDL cholesterol

The concentration of LDL cholesterol is calculated using the equation by Friedewald (Friedewald et al., 1972). Providing that the major lipoprotein fractions (total cholesterol, HDL cholesterol and triglycerides) are known, LDL cholesterol can accurately be calculated with the following formula:

$$\text{LDL cholesterol} = [\text{total cholesterol}] - ([\text{HDL}] + ([\text{triglycerides}] / 5))$$

3.3.2.2 Atherogenic Index of plasma

A triglyceride based index used by practitioners as a significant predictor of atherosclerosis, known universally as the atherogenic index of plasma (AIP) is calculated as follows:

$$AIP = \text{Log} \frac{(\text{Triglycerides}}{\text{HDL cholesterol})}$$

3.3.2.3 C - reactive protein

CRP has been implicated as a contributor to atherogenesis by modulating endothelial function, inducing the expression of VCAM-1, ICAM-1, E-selectin, stimulating coagulation, mediating uptake of low density lipoproteins into macrophages as well as destabilizing plaques (Volanakis, 2001). The C - reactive protein (human) EIA kit (item No. 10011236) was supplied by Cayman Chemical Company, Ann Arbor, MI. The kit supplied is an immunometric assay that is used to measure CRP in plasma without prior sample purification. The standard curve range is 0-3000 pg/mL and the limit of detection is about 50 pg/mL.

The assay is based on a double-antibody sandwich technique. Each well of the microwell plate is coated with monoclonal antibody which binds human CRP that is introduced to each well. Samples and standards are incubated on the anti-body coated microwell plate, which is then rinsed before adding HRP-labelled CRP monoclonal antibody for the detection of captured CRP. A sandwich is formed from the two antibodies through the binding on the CRP molecules at different locations. The analyte concentration is identified via measuring HRP enzymatic activity using the chromogenic substrate TMB (3,3',5,5'-

tetramethylbenzidine). The reaction is then stopped with acid after a sufficient period of time. In turn, this forms a product with a distinct yellow colour which is measured spectrophotometrically at 450 nm. The intensity of the colour measured is directly proportional to the amount of bound HRP-labelled monoclonal antibody, thus in turn is in proportion to CRP concentration.

CRP (human) assay buffer was prepared by the student researcher according to the manufacturer's instructions, by reconstituting the contents of the buffer packet with 1 L of ultrapure water. Plasma samples were diluted between the recommended range of 1: 1,000 and 1: 16, 000. Anti-CRP (human) HRP conjugate (100X) was diluted with 13 mL of assay buffer. A series of eight standards (3000, 1500, 725, 375, 187.5, 93.8, 46.9, 0 pg/mL) were prepared using a serial dilution of CRP standard with assay buffer as diluent. 100 µL of standard and diluted plasma samples were added to a 96-well microplate followed by an incubation of one hour at room temperature on an orbital shaker. All wells were then emptied and rinsed four times with assay buffer. 100 µL of anti-CRP (human) HRP conjugate was then added to each well except the blank wells. The plate was then incubated for 30 minutes at room temperature on an orbital shaker. All wells were then emptied and rinsed four times with assay buffer. 100 µL of CRP TMB substrate solution was then added to each well, followed by 15 minutes of incubation at room temperature in the dark. Finally, 100 µL of the CRP HRP stop solution was then added to each well and the plate was read at a wavelength of 450 nm with a microplate absorbance spectrophotometer. A standard curve was generated using Microsoft Excel and the plasma concentration of CRP was then calculated.

3.3.2.4 Insulin

The insulin ELISA (enzyme linked immunosorbent assay) kit was supplied by Mercodia AB, Sylveniusgatan, Sweden. The insulin ELISA is a solid phase two-site enzyme immunoassay. The procedure was performed by the student researcher and it is based on the direct sandwich technique, where two monoclonal antibodies are directed against separate antigenic determinants on the insulin molecule. At the time of incubation, insulin in the sample reacts with peroxidase-conjugated anti-insulin antibodies and anti-insulin antibodies bound to microplate wells. The washing step removes unbound enzyme labelled antibody. The bound conjugate is detected by reaction with TMB. By adding acid the reaction stops in turn giving a colorimetric endpoint which is measured spectrophotometrically at 450 nm.

The quantitative determination of human insulin in plasma was performed according to the manufacturer's instructions. No dilution was required for human plasma samples. 1.0 mL of enzyme conjugate 11X solution was diluted with 10 mL of enzyme conjugate buffer. 35 mL of wash buffer 21X solution was diluted with 700 mL of redistilled water. 25 µL of calibrators, controls and samples was added into the appropriate wells. 100 µL of enzyme conjugate 1X solution was added to each well, followed by incubation of the plate for one hour at room temperature on a plate shaker (700-900 rpm). All wells were then emptied and rinsed six times with wash buffer. 200 µl of substrate TMB was then added to each well, followed by plate incubation of 15 minutes at room temperature. Finally, the stop solution was added (50 µL) to each well and the plate was placed in the microplate absorbance spectrophotometer. The optical density was read at 450 nm and then a calibrator curve was generated using Microsoft Excel and the plasma concentration of insulin was then calculated.

3.3.2.5 Leptin

The leptin (human) ELISA kit was supplied by Enzo Life Sciences, Plymouth Meeting, PA, USA and the procedure was completed by the student researcher. The basic principle of the assay is as follows: Standards and samples are added to appropriate wells coated with monoclonal antibody specific for human leptin. The microplate is then incubated and then washed which leaves only bound human leptin on the microplate. A yellow solution of biotinylated polyclonal antibody specific for human leptin is then added, binding the human leptin captured on the plate. The plate in incubated and then washed to remove excess antibody. A blue solution of streptavidin conjugate to horseradish peroxidase (HRP) is then added to each well which binds to the biotinylated antibody. The plate is incubated and then washed to remove excess HRP conjugate. TMB substrate solution is then added and a HRP-catalyzed reaction produces a blue colour in the solution. The substrate reaction is then stopped by adding stop solution and the yellow colour is measured spectrophotometrically at 450 nm. A standard curve was generated using Microsoft Excel and the plasma concentration of leptin was then calculated. A summary of the assay process is presented in Figure 3.1.

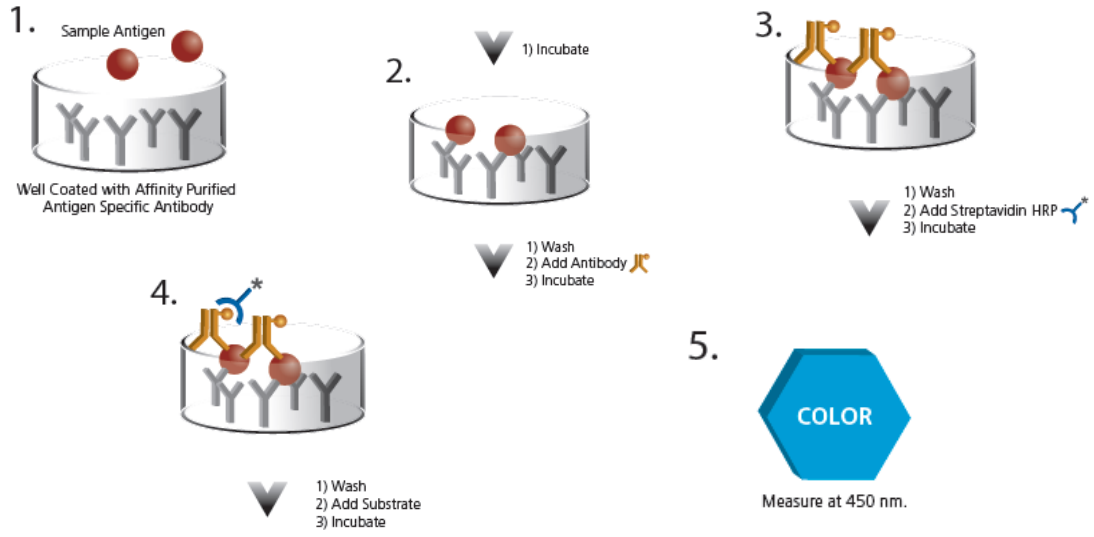


Figure 3.1: Schematic of the human leptin ELISA (Adopted from: Leptin (human) ELISA kit, Enzo Life Sciences, Plymouth Meeting, PA, USA.).

The quantitative determination of human leptin in plasma was performed according to the manufacturer's instructions. Plasma samples were diluted into assay buffer 17 at a 1: 50 dilution. 50 mL of wash buffer concentrate was diluted with 950 mL of deionized water. A series of seven standards (2000, 1000, 500, 250, 125, 62.5, 31.3 pg/mL) were prepared using a serial dilution of leptin standard with assay buffer as diluent. 100 µL of assay buffer was added to the standard 0 wells. 100 µL of standards and samples were added to the appropriate wells and the plate was incubated at room temperature for one hour. All wells were then emptied and rinsed four times with wash buffer. 100 µL of yellow antibody was added to each well except the blank and then the plate was incubated for one hour at room temperature. All wells were then emptied and rinsed four times with wash buffer. 100 µL of blue conjugate was added to each well except the blank and incubated for 30 minutes at room temperature. All wells were then emptied and rinsed four times with wash buffer. 100 µL of substrate solution was added to each well and then the plate was incubated for another 30 minutes at room temperature. Finally, the stop solution of 100 µL was added to each well and the plate was placed in the microplate absorbance spectrophotometer. The optical density was read at 450 nm and then a standard curve was generated using Microsoft Excel and the plasma concentration of leptin was then calculated.

3.3.2.6 Ghrelin

Total ghrelin concentrations were measured by radioimmunoassay (RIA) using Millipore's Ghrelin (Total) RIA commercially available kit (Saint Charles, MI, USA) utilizing ^{125}I-labelled ghrelin and a ghrelin antiserum to determine the level of total ghrelin in plasma. The assay procedure flow chart is presented in Table 3.6. To prepare the total ghrelin standard, it was reconstituted with 2 mL of distilled water and mixed gently until dissolved. A series of

six standards were prepared using a serial dilution of ghrelin standard with assay buffer as diluent. Total ghrelin quality control 1 & 2 were reconstituted with 1 mL of distilled water and then mixed gently.

The assay procedure was completed over three days. For the first day the following protocol was followed: 300 µL of assay buffer was added to the non-specific binding tubes (3-4). 200 µL of assay buffer was added to the reference tubes (5-6). 100 µL of assay buffer was then added to tubes seven through the end of the assay. Thereafter, 100 µL of standards, quality controls and sample were added in duplicate. 100 µL of ghrelin antibody was added to all tubes except total count tubes (1-2) and non-specific binding tubes (3-4), and were then vortexed, covered and left to incubate overnight (20-24 hours) at 4 °C.

For day two, the ^{125}I-ghrelin tracer was hydrated with 13.5 mL of label hydrating buffer, mixed gently and then 100 µL was added to all tubes, vortexed, covered and left overnight (22-24 hours) at 4 °C.

On day 3, 1.0 mL of cold (4 °C) precipitating reagent was added to all tubes except total count tubes (1-2), then vortexed and left to incubate for 20 minutes at 4 °C, followed by centrifugation at 4 °C for 20 minutes at 2,000-3,000 xg. The supernatant from all centrifuged tubes except total count tubes (1-2) were immediately decanted and drained for 15-60 seconds. The pellets were counted on a gamma counter according to the manufacturer's instructions. The final counts were downloaded from the gamma counter and then converted into Microsoft Excel format. Concentrations of total ghrelin were calculated using the "Assay Zap Universal Assay Calculator" (Elsevier-Biosoft, Cambridge, UK).

Table 3.6: Total ghrelin assay procedure flow chart (Adopted from: Millipore's total ghrelin RIA commercially available kit, Saint Charles, MI, USA)

Day 1					Day 2		Day 3	
Set-up	**Step 1**	**Step 2 & 3**	**Step 4**	**Step 5**	**Step 6**	**Step 7**	**Step 8**	**Step 9-11**
Tube number	Add assay buffer	Add standard/quality control/ sample	Add ghrelin antibody	Vortex, cover and incubate 20-24 hours at 4 ℃	Add 1-125 ghrelin tracer	Vortex, cover and incubate 22-24 hours at 4 ℃	Add precipitating reagent	Incubate 20 minutes at 4 ℃, centrifuge at 4 ℃ for 20 minutes, decant and count
1,2	-	-	-		100 µL		1.0 mL	
3,4	300 µl	-	-		100 µL		1.0 mL	
5,6	200 µL	-	100 µL		100 µL		1.0 mL	
7,8	100 µL	100 µL of tube 6	100 µL		100 µL		1.0 mL	
9,10	100 µL	100 µL of tube 5	100 µL		100 µL		1.0 mL	
11,12	100 µL	100 µL of tube 4	100 µL		100 µL		1.0 mL	
13,14	100 µL	100 µL of tube 3	100 µL		100 µL		1.0 mL	
15,16	100 µL	100 µL of tube 2	100 µL		100 µL		1.0 mL	
17,18	100 µL	100 µL of tube 1	100 µL		100 µL		1.0 mL	
19,20	100 µL	100 µL of reconstitute	100 µL		100 µL		1.0 mL	
21,22	100 µL	100 µL of quality control 1	100 µL		100 µL		1.0 mL	
23,24	100 µL	100 µL of quality control 2	100 µL		100 µL		1.0 mL	
25,26	100 µL	100 µL of unknown	100 µL		100 µL		1.0 mL	

3.4 Experimental outline of animal study

3.4.1 Animals and housing

Male Wistar rats (mean weight: 211.5 g; weight range: 207.8 – 213.4 g; 4 weeks old) supplied from the Animal Resources Centre, Perth, Australia were used for this experiment. The animals were transported by overnight road-air link from the supplier. During transportation, the animals were group-housed in ventilated boxes supplied with bedding. The rats were held in the animal facility of the Howard Florey Institute, The University of Melbourne. The Animal Ethics Committee of the Howard Florey Institute, University of Melbourne approved the experimental protocol (AEC 11-037) and all handling and management of procedures were carried out in accordance with the ethical principles and regulations specified by the *Prevention of Cruelty to Animals Act 2004* and the *NHMRC Australian Code of Practice for the Care and Use of Animals for Scientific Purposes* (Seventh Edition, 2004).

The rats were housed in individual solid bottom polypropylene cages to allow for individual measurement of the ingestion of the *C. fimbriata* extract. Wood shavings were used as the bedding material which was changed once per week and the cages were provided with *ad libitum* of tap water and the group-specific rat diet. The animals were housed in an environmentally controlled laboratory with a seven day acclimatization period prior to commencement of the trial. During acclimatization, rats were given a standard pellet chow (Specialty Feeds, Western Australia) and water *ad libitum*. The animal facility was under a 12 hour light – 12 hour dark cycle (lights off at 19.00 hours), at a constant temperature of 22 °C ± 2 °C and relative humidity of approximately 60 %. To monitor the well-being of the animals, body weight and food intake was measured daily and well groomed fur was used as a marker of well-being. Rats were habituated to everyday handling by humans.

3.4.2 Administration of Caralluma fimbriata extract

The *C. fimbriata* extract was sourced from Gencor Pacific group (Hong Kong) and imported by AZPA, Keilor Park, Melbourne, Australia. The *C. fimbriata* extract is a patented and proprietary hydroethanolic extract produced by extracting the dry aerial parts of the plant with 40 % alcohol to attain no less than 25 % pregnane glycosides and saponin glycosides. To increase the shelf life and uniformity, the extract was then lyophilised completely by a continuous freeze-drying operation to produce a brown powder.

3.4.3 Experimental design

Male Wistar rats (4 weeks old) were divided into four groups, with 10 rats in each group (n = 40) by the student researcher. For randomisation, rats were allocated into four groups based on their body weight and usual food intake. The study was over a 15 week period: the initial seven weeks to induce obesity and the following eight weeks for *C. fimbriata* extract treatment. For the first seven weeks, the lean rats in group one and three (without *C. fimbriata* extract) were fed a standard pellet chow (Barastoc rat and mouse feed, Ridley AgriProducts, Victoria, Australia), while group two and four (without *C. fimbriata* extract) were fed a high fat diet (SF00-219, Cholesterol semi-pure rodent diet, Specialty Feeds, WA, Australia). It was expected by seven weeks that group two and four would present with obesity (Buettner et al., 2007). Treatment with *C. fimbriata* extract was given to groups three and four for an eight week intervention period. Groups two and four still continued with the high fat diet. The *C. fimbriata* extract was administrated in the form of powder (100 mg/kg BW per day). A previous study also used this dose (Kamalakkannan et al., 2010). The *C.*

fimbriata extract was mixed in with a peanut butter vehicle upon feeding to improve palatability. Control rats received the peanut butter only.

3.5 Metabolic measurements of animal study

3.5.1 Body weight and food consumption

Body weight (g) was recorded daily with digital scales (TE4101, Precision Balance Sartorius AG, Goettingen, Germany). Daily food consumption was identified by weighing feed clearance. The calculated nutritional parameters of the high fat diet and standard chow diet are presented in Table 3.7.

Table 3.7: Calculated nutritional parameters of animal fed diets

Nutritional parameters	High fat diet	Standard chow diet
Protein (%)	19.0	20.0
Total fat (%)	21.0	5.0
Crude fibre (%)	4.7	5.0
Digestible energy (MJ/kg)	19.4	17.25
Digestible energy from lipids (%)	40.0	10.7
Digestible energy from protein (%)	17.0	19.7

3.5.2 Body composition

Abdominal circumference was assessed at the end of the study when the rodent was anaesthetized with the rat placed in the ventral position. Abdominal circumference was taken on the largest zone of the rat abdomen using a plastic non-extensible measuring tape with an accuracy of 0.1 cm.

Whole body composition (fat mass, lean mass, free water and total body water) was determined in conscious rats by using the EchoMRI-900 body composition analyser (EchoMedical systems, Houston, Texas, USA) at pre-intervention and post-intervention. Calibration of the machine was completed according to the manufacturer's instructions using canola oil as the calibration medium. The body scan was completed in duplicate to ensure accuracy of measurements and the mean of the two scans was used as the final reading. Data were exported to Windows XP Professional Edition (Microsoft).

Following the treatment period, rats were anaesthetised and abdominal adipose tissue was dissected and weighed. Visceral fat mass was determined by weighing the left perirenal and epididymal adipose fat pads.

3.5.3 Blood pressure

Systolic blood pressure and diastolic blood pressure values were measured in conscious, pre-warmed, restrained animals by non-invasive tail-cuff plethysmography (CODA Non-invasive blood pressure System for rats, Kent Scientific Corporation, Connecticut, USA) at baseline and at the end of the supplementation period. To increase tail vein circulation, rats were pre-warmed in a heating chamber for 10 minutes at 30 °C. The tail cuff and volume pressure sensor were placed on the tail of each rat and then underwent five acclimatisation cycles followed by 10-20 cycles of cuff inflation (5 seconds between cycle sets). Systolic and diastolic blood pressure readings were analysed using CODA software.

3.5.4 Urine collection

To monitor possible effects on kidney function, 24-hour urine collection was taken at 3-time points during the experiment. Metabolic cages were used to collect the rodent's urine over a 24 hour period at baseline and at the end of the supplementation period. One rat was placed in each metabolic cage provided with a wire mesh bottom and a funnel to collect urine. Urine was collected into pre-weighed plastic containers to identify total volume of urine voided. Rats were provided with their group specific feed and tap water *ad libitum*. After 24 hours of collection, the urine samples were transported to the laboratory on ice immediately after collection. The urine was then centrifuged to remove solid debris such as food, faecal matter or fur at 4000 x g (RCF 3399) at 4 °C for 10 minutes. Urine was then collected into 2 mL Eppendorf tubes and frozen at -80 °C for further analysis.

3.5.5 Urinary sodium excretion

Urinary sodium content was measured at baseline and post-intervention using the flame photometer (Corning Clinical Flame Photometer 410c, Corning 805 Dilutor, Sherwood Scientific Limited, Cambridge, United Kingdom). The flame photometer was calibrated according to the manufacturer's instructions. Undiluted samples were used to measure sodium content and were expressed as mmol/L.

3.5.6 Insulin sensitivity

Animals were deprived of food for at least 2 hours but were allowed to drink tap water. The animal's weight was recorded to determine the insulin dosage. Rats were given 0.75 U/kg

body weight of insulin (Humalog, Eli Lilly, NSW, Australia, Pty Ltd). The insulin solution was prepared with 50 µL of insulin and 20 mL of 0.9 % of saline.

Following the two hour fast, the baseline blood glucose was measured with a blood glucose meter kit (Optium Freestyle blood glucose meter kit, Abbott Laboratories Limited, England) via the tail snip method. The tail tip of each rat was clipped to obtain blood (approximately 25 µL) for blood glucose determination (mmol/L). The insulin solution was administrated when the rat was placed in a towel roll. The insulin was injected intraperitoneally into the abdominal cavity just below the abdominal muscle layers. Blood glucose was monitored at 15, 30, 60, 90 and 120 minutes following administration of insulin. At the completion of the test, all rats were given food and water. The insulin sensitivity test was completed at baseline and at the end of the supplementation period.

3.5.7 Glucose tolerance

Two days following the insulin tolerance test, an intraperitoneal glucose tolerance test (IPGTT) was conducted. The rodents were fasted for 24 hours prior to the commencement of the test. Animals were weighed to determine the volume of glucose to be injected. Before the glucose load, a fasting measure was obtained using the blood glucose meter kit (Optium Freestyle blood glucose meter kit, Abbott Laboratories Limited, England). The glucose load (2 g/kg BW) was administrated via an intraperitoneal injection. Blood was monitored via the tail snip method post IP by reopening the initial incision at 15, 30, 60, 90, 120 minutes and a commercial glucose meter (MediSense, Precision Plus, Abbott Diabetes Care Inc.) was used to determine the blood glucose level. The IPGTT was performed at baseline and at the end of the supplementation period.

3.6 Blood sample collection

Following the end of the treatment period, rodents were deeply anaesthetised by intraperitioneal injection at a dose of 100 mg/kg BW with sodium Pentobarbitone. A laparotomy incision was made once the animals were anaesthetised for the gross examination of abdominal tissues and blood collection. Rats were euthanized following cardiac puncture through which blood samples of approximately 10 mL were collected from the heart into heparinised tubes and chilled on ice. Plasma was obtained by blood centrifugation at 4000g at 4 °C for 10 minutes, aliquoted and stored at -80 °C for further analysis.

3.7 Plasma analysis

3.7.1 Total cholesterol and triglyceride analysis

Rat triglyceride levels and total cholesterol were measured by enzymatic methods, using commercially available kits (Thermo Fisher Scientific Inc, Middletown, USA). Measurement of total cholesterol and triglycerides has been described in detail in sections 3.3.2.1.1 and 3.3.2.1.2.

3.7.2 Plasma glucose

The glucose analyser (YSI 2300 STAT Plus) was used to analyse post intervention plasma glucose (mmol/L) levels. The machine was calibrated according to the manufacturer's instructions.

3.8 Tissue collection and analysis

3.8.1 Liver lipid content

The current protocol for lipid extraction is the Folch procedure (Folch et al., 1957). Liver was dissected into finely sliced 3g pieces and placed in a solution containing 50 mL of chloroform (LiChrosolv, Merch, Damstadt, Germany): methanol (LiChrosolv, Merch, Damstadt, Germany) 2:1 ratio, containing approximately 10 mg/L butylated hydroxytoluene (Kosher, SAFC, Sigma-Aldrich, Louise, USA) (antioxidant used for longevity of samples) and were kept in the cool room.

For lipid extraction, the samples were returned back to room temperature. The extraction solution was filtered into a separation funnel equipped with filtration paper. Furthermore, the sample bottles were rinsed with 10 mL of chloroform: methanol (2:1 ratio) twice and were poured through the funnel with the filtration paper into the separation funnel. Then the bottles were rinsed with 10 mL of chloroform once. By rinsing the bottles this permitted maximum collection of the extraction solution. Then the funnel and filter paper was removed when the greatest amount of the extraction solution was in the separation funnel and the filter paper was dry. Next, 16 mL of 0.6 % NaCl (2.4g sodium chloride powder and 400 mL of distilled water H_2O) was poured into the separation funnel. The separation funnels were capped, mixed well and left overnight at room temperature to allow the solutions to partition.

Once the upper and lower phase of the solutions was reached, the bottom phase containing the lipid was collected in a round bottomed flask (pre rinsed with chloroform) and the upper phase was discarded. In addition, rotary evaporation (Heidolph VV 2000, waterbath type WB 2000, Schwabach, Germany) at 40 °C, speed 120-150 with warm water of the bottom phase

occurred. The rotary evaporator rotates the round flask which causes the solvent to evaporate and therefore the lipid is left in the round flask.

Once dry, the lipid was reconstituted with 2.5 mL of chloroform ($CHCI_3$) three times to ensure the maximum amount of lipid extract was obtained. Then the lipid extract was transferred into a 10 mL volumetric flask up to the mark and mixed well with the pipette. Then 1 mL of the lipid extract was transferred into a pre-weighed 4 mL vial, dried off under N_2 gas via the nitrogen evaporator (N-EVAP111, Organomation Associates Inc., Berlin, MA, USA) (leaving only the lipid) and left in the desiccator overnight (lidless) to prevent moisture exposure. The 4 mL vials placed in the desiccator were re-weighed to calculate the lipid (g/mL) content. The total lipid as percentage to the liver wet tissue was calculated with the following formula:

Lipid in 1 mL solution x (10 / 3) x 100*

*10 = volume of lipid extract. *= mass of tissue sample acquired from dissection.*

3.8.2 Histological analysis of liver

Liver tissue was sectioned, weighed and finely diced using a sharp scalpel blade. Liver samples were fixed in 10 % neutral-buffered formalin (NBF). The formalin solution was prepared by mixing 100 mL of formaldehyde (Ajax Finchem Pty Ltd, NSW, Australia), 900 mL of distilled water, 4.0 g of NaH2P04 (Sodium dihydrogen orthophosphate dehydrate, BDH Laboratory Supplies, England) and 6.5 g of Na_2HPO_4 (Sodium phosphate, Sigma Chemical Co, St Louise, USA) together. Liver tissue was then dehydrated via a series of graded ethanol baths in order to displace the water and was then infiltrated with wax. This was achieved by firstly submerging liver tissue in 70 % ethanol to begin the processing

schedule using the automated tissue processor for a total of 14 hours. The following paraffin processing cycle was used: 70 % ethanol for 1 hour, 90 % ethanol for 1 hour, 100 % ethanol for 1 hour, 100 % ethanol for 1 hour and 30 minutes, 100 % ethanol for 1 hour and 30 minutes, 50/50 % ethanol/xylene for 1 hour and 30 minutes, xylene for 1 hour and 30 minutes, paraffin wax for 1 hour, paraffin wax for 1 hour, paraffin wax for 1 hour, and paraffin wax for 30 minutes.

Following embedding of tissue into wax blocks (stored at room temperature), microtome sectioning was then undertaken. Firstly, the water bath was set at 47-48 °C with fresh ionized water and the slide warmer was set at 60 °C. The blocks that were sectioned were first placed face down on an ice block for at least 20 minutes to keep the tissue cool. A fresh blade was placed on the microtome after sectioning up to 10 blocks. The rotary handle was locked and the guard was then put over the blade. The alignment of the blade was set at 10. To begin, the paraffin block (cassette) was put on the clamp (specimen holder) with the block facing the blade and aligned in a vertical plane. The microtome was then unlocked. The distance between the paraffin block and the blade was adjusted so that the blade was only touching the block slightly by turning both wheels. The microtome dial was set at 20-30 microns (thickness of trim) in order to plane the block. Once the blade was cutting smoothly, the dial was set at 2 microns. Once the desired section of thickness was produced, the ribbon was transported to the water bath (floating on the surface of the water bath). A microscope slide was put at a 90 °C angle next to the section and then pulled up (the section adhered to the glass slide). The slides were put in a pre-labelled rack and put in the oven (37 °C) to bond the tissue to the glass ready for Haematoxylin and Eosin (H & E) staining.

H & E staining was performed following a standard protocol (Ross and Pawlina, 2011). Sections were cleared in xylene for five (3 and 2 minutes) minutes, then immersed in absolute

alcohol for two minutes (1 and 1 minute) and rinsed in 70 % alcohol and then rinsed in 30 % alcohol followed by a thorough wash with tap water. Sections were then counterstained with Mayer's haematoxylin for two minutes, then returned to tap water and then immersed in Scott's tap water (blueing solution) for one minute and then rinsed in tap water again for one minute. The sections were then examined microscopically. Following this, sections were then stained with Eosin (1 % Sigma Aldrich) for two minutes followed by a brief rinse in tap water. Sections were then immersed in 30 % alcohol (x 10 dips), then 70 % alcohol (x 10 dips) and then absolute alcohol for 30 dips (10 dips and 20 dips) and then cleared with xylene for 3 minutes (1 and 2 minutes) and mounted with DPX mounting media (Thermo Scientific) on coverslips. Liver sections were imaged at 400 X magnification (Carl Zeiss microscope) from two rats in each group for the analysis of liver lipid droplets using Axio Vision 4.8 software.

3.9 Quantification of results and statistical analysis

The sample size for study 1 & 3 (a minimum of 13 per group) was determined by statistical power analysis (using the mean change of 25 and standard deviation of 20) (two tailed t-test at the 0.05 significance level for the power of 90 % of expected differences in appetite, one of the major measured outcomes) based on the previous findings of Kuriyan et al (2007). All data were expressed as mean ± SEM, unless specified otherwise. For study 1, a one-way ANOVA and multiple comparisons using a Tukey HSD post hoc analysis were performed to assess the change (expressed as delta) in the parameters over time between groups and within the group. For study 2, one-way ANOVA was performed to compare all baseline data between groups. A mixed model analysis was carried out to compare four groups and where the main effects were significant ($p < 0.05$), means were compared using Tukey's multiple comparisons test. For study 3, mixed model analysis was also used to analyse the effects of the intervention, time, and the interaction between the intervention and time with pairwise comparisons (adjusted for multiple comparisons by Bonferroni's post-hoc test). When the interaction and/or the main effects were significant, means were compared using Tukey's multiple comparison post hoc test. P values were accepted at $p < 0.05$. Statistical analysis was performed and 95 % confidence intervals (CI) was calculated using the SPSS package, version 22 (SPSS, Chicago, IL, USA).

CHAPTER 4: STUDY 1 - A PILOT STUDY INVESTIGATING THE EFFECT OF CARALLUMA FIMBRIATA EXRACT ON THE RISK FACTORS OF METABOLIC SYNDROME IN OVERWEIGHT AND OBESE SUBJECTS: A RANDOMISED CONTROLLED CLINICAL TRIAL

ASTELL, K. J., MATHAI, M. L., MCAINCH, A. J., STATHIS, C. G. & SU, X. Q. 2013. A pilot study investigating the effect of *Caralluma fimbriata* extract on the risk factors of metabolic syndrome in overweight and obese subjects: a randomised controlled clinical trial. *Complement Ther Med*, 21**,** 180-9.

4.1 Summary

Objectives: Central obesity is a key component of metabolic syndrome and it is often associated with other risk factors such as dyslipidemia, elevated plasma glucose levels and elevated blood pressure (BP). In this pilot study, the effect of *Caralluma fimbriata* (an edible succulent) extract in combination with controlled dietary intake and physical activity on these risk factors was assessed in overweight and obese Australian subjects compared to placebo.

Design: This was a randomized, double blind placebo controlled clinical trial. Forty three adults aged 29-59 years were recruited. The eligibility criteria included a BMI > 25 kg/m^2, or a waist circumference > 94 cm (male), > 80 cm (female). Thirty three participants completed the 12-week study at Victoria University, Nutritional Therapy Clinic. Participants were randomly assigned into two groups. *Caralluma fimbriata* extract and placebo were orally administered as 500 mg capsules twice daily (1g/day) and dietary intake and exercise were monitored weekly.

Results: The results of thirty three participants (experimental group, n = 17; placebo group n = 16) were analysed. The primary outcome measure was the decline in waist circumference. By week 9, the experimental group had lost 5.7 cm, compared to only 2.8 cm loss in the placebo group (Difference: -2.890; 95 % CI; -5.802 – 0.023). Post intervention, the experimental group had lost 6.5 cm compared to 2.6 cm loss in the placebo group (Difference: -3.847; 95 % CI; -7.466 – 0.228). WHR also improved significantly after 12 weeks intervention in the experimental group, with a total reduction of 0.03 being recorded compared to 0.01 increase in the placebo group (Difference: -0.033; 95 % CI; -0.064 – -0.002). There was also a significant decline in the palatability (visual appeal, smell, taste) of the test meal and sodium intake in the experimental group at week 12 ($p < 0.05$). In addition a significant reduction in body weight, BMI, hip circumference, systolic BP, HR, triglyceride levels, total fat and saturated fat intake within both groups was observed following the intervention period ($p < 0.05$).

Conclusion: Supplementation with *Caralluma fimbriata* extract whilst controlling overall dietary intake and physical activity may potentially play a role in curbing central obesity, the key component of metabolic syndrome. Controlling dietary intake and exercise improved body weight and favourably influenced the metabolic risk profile.

Name of trial registry: Australian and New Zealand Clinical Trials Registry

Registration number: ACTRN12610000289011

4.2 Introduction

Metabolic syndrome is a complex disorder characterized by a clustering of cardiovascular risk factors including abdominal/central obesity, dyslipidemia, elevated plasma glucose levels and elevated BP (The National Cholesterol Education Program, 2001). Central obesity is one of the major determinants of metabolic syndrome (Carr et al., 2004). Pathological mechanisms involved in metabolic syndrome include ectopic lipid accumulation resulting in lipotoxicity and altered secretion of adipocytokines (adipocyte-derived hormones) (Carr et al., 2004). Visceral fat is metabolically active as a source of adipocytokines chiefly leptin (Yun et al., 2010), adiponectin (Yatagai et al., 2003), plasminogen activator inhibitor type 1 (Giltay et al., 1998), tumour necrosis factor alpha (van Harmelen et al., 2002) and non-esterified fatty acids (Abate et al., 1995).

The majority of individuals affected by metabolic syndrome are overweight or obese, thus dietary treatment is focused on weight reduction (Grant and Meigs, 2005). Strategies for body fat reduction typically involve a combination of lifestyle changes such as limiting calorie intake, increasing physical activity, behavioural therapy, pharmacotherapy, and surgery (Celleno et al., 2007).

The availability and popularity of natural dietary supplements for weight loss has risen dramatically in recent years. Among potential natural supplements for weight reduction are the appetite suppressants. One such supplement is the extract of *Caralluma fimbriata,* an edible succulent plant, in the Asclepiadaceae family, native to India (Kuriyan et al., 2007). Indian tribal people have used the natural appetite suppressant for many centuries, and in times of famine it is a commonly used vegetable (Kuriyan et al., 2007). The appetite

suppressing properties of *C. fimbriata* has been attributed to the active component, pregnane glycosides (Kunert et al., 2008). The mechanism of appetite suppression by pregnane glycosides is unclear, however one hypothesis is that *C. fimbriata* may down-regulate ghrelin synthesis in the stomach and neuropeptide-Y in the hypothalamus, resulting in appetite suppression (Gardiner et al., 2005).

Preliminary human clinical trials have shown significant weight reductions in overweight Indian subjects with supplementation of *C. fimbriata* extract in addition to lifestyle modification (Lawrence and Choudhary, 2004). A study by Kuriyan et al (2007) on the appetite suppressing effects of *C. fimbriata* in overweight Indian adults (25-60 years) showed a significant reduction in waist circumference after two months intervention. In addition the hunger level of participants reduced by 20 % which may account for an 8 % decline in energy intake of the experimental group (Kuriyan et al., 2007). However, Kuriyan et al (2007) did not identify a significant reduction in blood lipid profile in the subjects with or without *C. fimbriata* supplementation. Also, no human trials have reported the effect of *C. fimbriata* extract on other metabolic risk factors including plasma glucose levels, BP and adipocytokines such as leptin. The aim of this study was to determine whether *C. fimbriata* extract, in addition to a hypocalorie diet (deficit of 500kcal/day of estimated energy requirements) and regular physical activity, can attenuate metabolic disturbances including central obesity, elevated BP, dyslipidemia and elevated blood glucose levels in generally healthy and obese Australian adults.

4.3 Methods

4.3.1 Experimental design, participant recruitment and randomisation

This study was a randomized, double blind, placebo controlled clinical trial. It was conducted at Victoria University, Nutritional Therapy Clinic, Melbourne, Australia. Recruitment of participants and eligibility criteria are described in section 3.1 of this thesis. Thirty three volunteers (29-59 years), were randomly assigned (Figure 4.1) into either the placebo (n = 16; 14 females, 2 males) or the experimental group (n = 17; 12 females, 5 males).

The study was approved by the Human Research Ethics Committee of Victoria University, Australia (HRETH 10/22) and registered by ANZCTR. At the beginning of the study all eligible volunteers were informed about the details of the study including that they would be randomly assigned into one of the two groups (experimental or placebo) prior to signing the consent form.

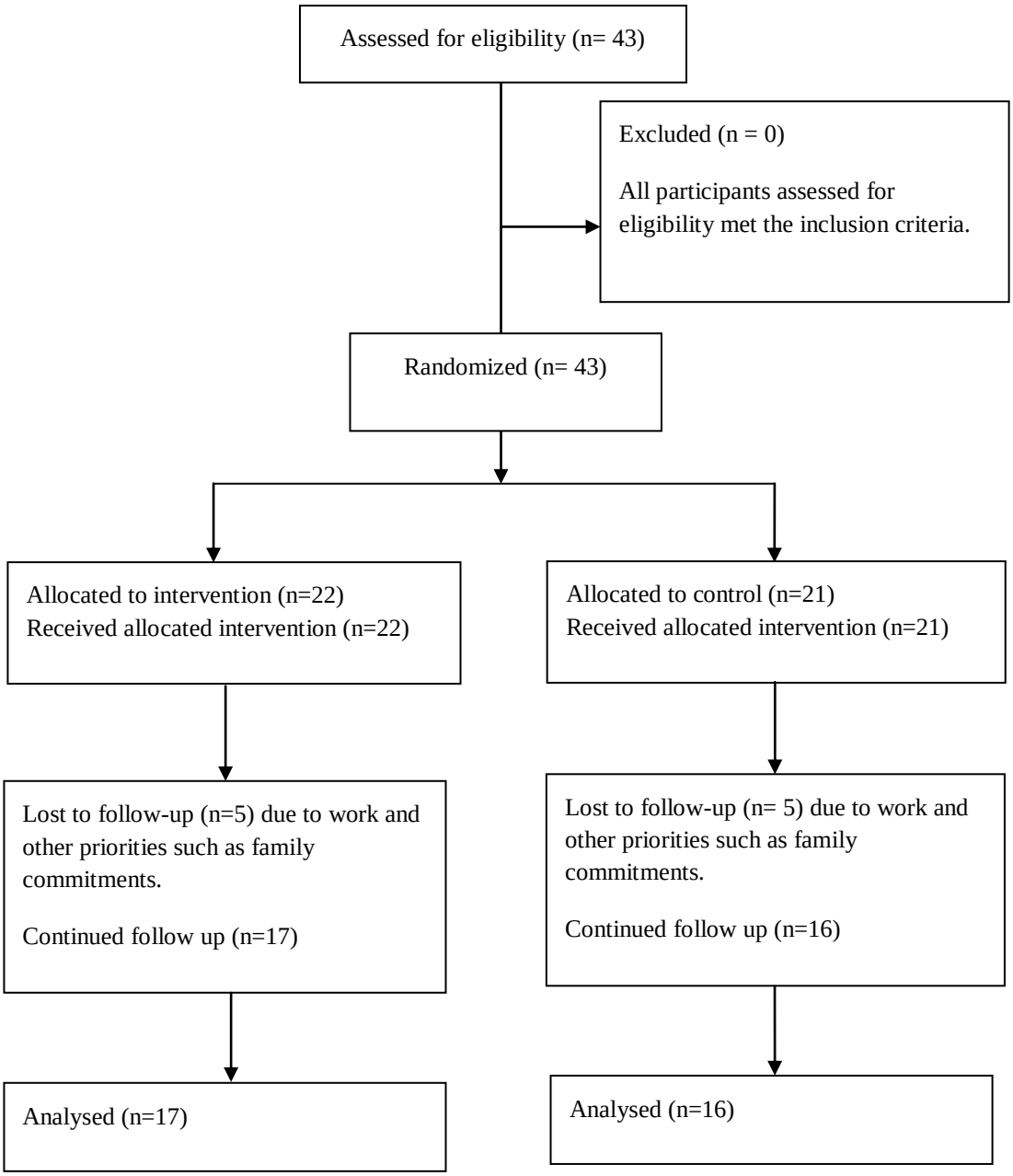


Figure 4.1: The study flow chart

4.3.2 Outcome measures

Prior to intervention, the participants completed a medical and a health and wellbeing questionnaire (Your Health and Wellbeing; SF-36v2 Health Survey). The baseline assessment included anthropometry (as described in section 3.2.1), metabolic parameters

(section 3.3), dietary intake (section 3.2.4), appetite (section 3.2.3), BP and heart rate (HR) (section 3.2.2). Appetite sensations were measured via the VAS method. Participants were required to record their appetite sensations for 'hunger', 'desire to eat', and 'fullness of stomach (satiety)' as well as rating the palatability of the meal on a scale of 0 to 100 mm.

The control capsule was a 500 mg capsule containing 100 % maltodextrin. The *C. fimbriata* extract and placebo capsules were provided in a 2-piece hard shell capsule form and delivered in blindly labelled sealed bags as 500 mg capsules twice daily (1 g/day) before meals for 12 weeks. This dosage was determined based on the previous study by Kuriyan et al (2007). Both capsules were supplied by AZPA Pharmaceuticals Pty Ltd, Melbourne, Australia. The ingestion of the capsules was monitored weekly during the nutrition consultations and a capsule calendar was also administered at the beginning of the trial and submitted at the completion of the 12 weeks.

During the intervention period dietary intake and exercise were controlled. Both groups received consistent dietary and exercise advice once per week. In order to obtain optimal compliance among participants, it was deemed helpful to provide and control participant's dietary and physical activity level. The dietary intake was monitored through 3-day food diaries fortnightly and participation in physical activity was also noted on the food diary submitted. The participants followed a hypocaloric diet (deficit of ~500 kcal/day of estimated energy requirements). Anthropometric measurements and BP were taken during each consultation. Appetite sensations were assessed at week six and three-day food diaries were collected fortnightly to monitor dietary intake.

After the 12-weeks intervention, anthropometry, BP, heart rate, appetite, dietary intake and biochemical parameters including blood lipid profile and plasma leptin levels were assessed. The food diaries were analysed using Food Works Professional 2009, version 6 (Xyris Software, QLD, Australia Pty Ltd) (Xyris Software Pty Ltd, 2007).

4.4 Statistical analysis

The sample size for the trial (a minimum of 13) was determined by statistical power analysis, two tailed t-test at the 0.05 significance level for the power of 90 % of expected differences in the major measured variable of the experiment i.e. appetite (using the mean change of 25 and standard deviation of 20) (Kuriyan et al., 2007). A 10 % reduction in appetite was considered significant, based on the previous study conducted by Kuriyan et al (2007). All data were expressed as mean ± standard deviation. Changes in each parameter between the two groups at various time points are expressed as magnitudes (delta). A one-way ANOVA and multiple comparisons using a Tukey HSD post hoc analysis were performed to assess the change in the parameters over time within the group and between groups. P values of less than 0.05 ($p < 0.05$) were considered statistically different. Statistical analysis was performed and 95 % CI was calculated using the SPSS package, version 19 (SPSS, Chicago, IL, USA).

4.5 Results

All screened volunteers met the inclusion criteria (n = 43). Ten participants (five in the experimental and five in the placebo group) did not complete the trial due to work and family commitments. These participants were excluded from the study (Figure 4.1). In addition, there was no breach of the blinding process identified throughout the intervention period.

The physical characteristics of the participants including age, body weight, BMI, waist and hip circumference and WHR were not significantly different between the two groups at baseline (Table 4.1).

Table 4.1: Physical characteristics of the subjects at baseline

Parameter	Experimental group (n = 17)	Placebo group (n = 16)
Age (yrs)	46.7±9.7	46.4±10.4
Body weight (kg)	93.0±16.5	91.8±15.3
Body mass index (kg/m²)	32.5±6.4	31.8±4.1
Waist circumference (cm)	102.1±11.2	100.3±12.1
Hip circumference (cm)	117.6±12.8	116.1±6.9
Waist to hip ratio	0.87±0.06	0.86±0.08

Values are expressed as means ± standard deviation (SD), n = number of subjects.

The primary outcome measure of this study was the decline in waist circumference. By week 9, the experimental group had lost 5.7 cm, compared to only 2.8 cm loss in the placebo group (Difference: -2.890; 95 % CI; -5.802 – 0.023). Post intervention, the experimental group had lost 6.5 cm compared to 2.6 cm loss in the placebo group (Difference: -3.847; 95 % CI; -7.466 – 0.228) (Figure 4.2).

WHR also improved significantly after 12 weeks intervention in the experimental group, with a total reduction of 0.03 being recorded compared to 0.01 increase in the placebo group (Difference: -0.033; 95 % CI; -0.064 – -0.002). There were no significant differences between the experimental and placebo group for anthropometry and blood pressure over the intervention period (Table 4.2). However there were significant within group changes observed compared to baseline data. Both groups showed a significant reduction in body weight, BMI and hip circumference after 12 weeks intervention ($p < 0.05$). Furthermore a significant reduction in systolic blood pressure and heart rate was also recorded in both groups ($p < 0.05$) (Table 4.2).

Table 4.2: Anthropometry, BP & HR at baseline, week 6 and week 12

Parameter	Experimental	Placebo	Difference [a] (95 % CI)
Body weight (kg)			
Baseline	93.0±16.5	91.8±15.3	
Week 6	91.1±16.3	89.8±16.1	0.191 (-1.18 – 1.56)
Week 12	91.0±16.6[b]	89.0±16.1[b]	0.787 (-1.85 – 3.42)
BMI (kg/m²)			
Baseline	32.5±6.4	31.8±4.1	
Week 6	32.1±6.5	31.2±4.2	0.213 (-0.41 – 0.84)
Week 12	31.9±6.2[b]	30.9±4.2[b]	0.349 (-0.41 – 1.10)
Waist circumference (cm)			
Baseline	102.1±11.2	100.3±12.1	
Week 6	96.8±9.7	95.8±12.6	0.733 (-4.00 – 2.53)
Week 12	95.6±10.1[b]	97.7±12.7[b, c]	-3.847 (-7.47 – 0.23)
Hip circumference (cm)			
Baseline	117.6±12.8	116.1±6.9	
Week 6	114.6±13	113.6±5.7	0.498 (-2.53 – 1.53)
Week 12	114.1±12.7[b]	112.1±6.1[b]	0.561 (-2.18 – 3.33)
WHR			
Baseline	0.87±0.1	0.86±0.1	
Week 6	0.85±0.1	0.84±0.1	0.004 (-0.029 – 0.04)
Week 12	0.84±0.1[b]	0.87±0.1[b, c]	-0.033 (-0.06 – -0.00)
Systolic Blood pressure (mm Hg)			
Baseline	126.4±11.4	135.3±26	
Week 6	120.6±15.8	125.2±19.5	4.421 (-3.10 – 11.95)
Week 12	120.1±15.4[b]	122.1±14[b]	7.015 (-4.39 – 18.42)
Diastolic blood pressure (mm Hg)			
Baseline	86.6±9.8	88.9±15.9	
Week 6	84.9±12.2	86.5±12.3	0.640 (-5.02 – 6.30)
Week 12	84.1±15.5	84.4±8.6	2.031 (-5.22 – 9.28)
Heart rate (Beats/min)			
Baseline	69.8±10.4	76.9±16.1	
Week 6	68.5±10.2	68.4±10.2[c]	7.175 (1.56 – 12.78)
Week 12	65.3±8.9[b]	70.1±11.3[b]	2.309 (-3.25 – 7.87)

Values are expressed as means ± SD. [a] Treatment minus Placebo. [b] A significant difference was observed at week 12 compared to baseline data ($p < 0.05$). [c] There was a significant change between groups. Exclusion of the value zero of the 95 % CI implies statistical significance at the 5 % level.

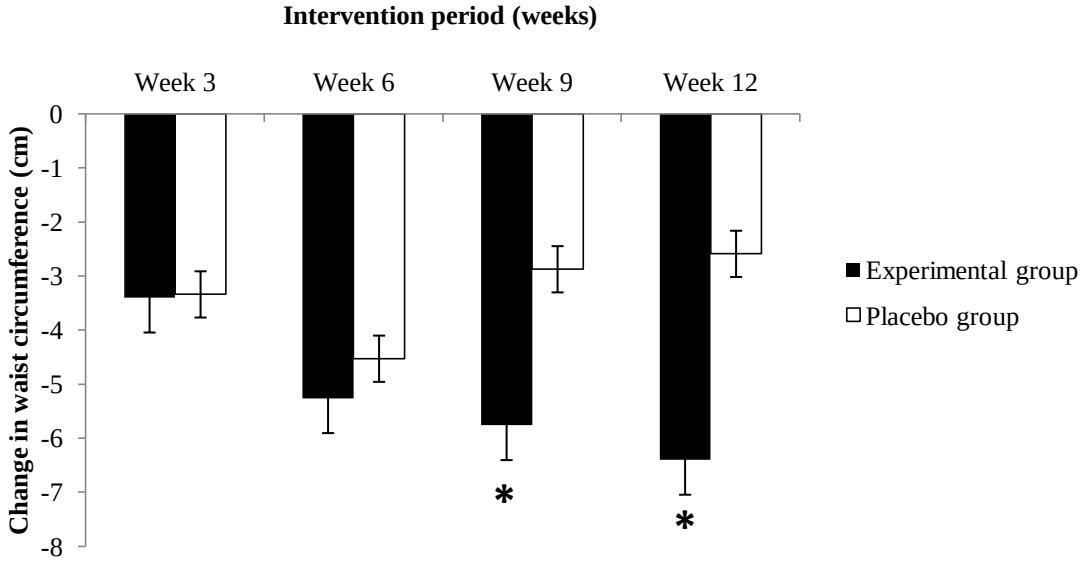


Figure 4.2: Change in waist circumference over the intervention period. * Significant difference between the two groups at week 9 (Difference: -2.890; 95 % CI; -5.802 – 0.023) and week 12 (Difference: -3.847; 95 % CI; -7.466 – 0.228).

The metabolic parameters of the two groups are shown in Table 4.3. There were no significant differences in metabolic parameters between the two groups over the intervention period. However, there was a significant reduction in triglyceride levels after 12 weeks intervention in both groups compared to baseline data ($p < 0.001$). The health and wellbeing survey results showed an overall improvement in general health in both groups. The 3-day food diary analysis (Table 4.4) showed a significant decline in total fat and saturated fat intake in both the experimental and placebo groups ($p < 0.05$). In addition, sodium intake in the experimental group was significantly reduced from 3.4 g to 2.2 g after 12 weeks intervention ($p < 0.05$).

The data on appetite assessment using the VAS method (Table 4.5) showed a significant reduction in the palatability (visual appeal, smell, taste) of the test meal in the experimental group compared to baseline data ($p < 0.05$). Although the placebo group showed a reduction

in desire to eat at week 6 (Difference: 0.625; 95 % CI; -1.45 – 2.70) (post ingestion) and week 12 (Difference: 0.856; 95 % CI; -1.56 – 3.27) (before ingestion), and hunger (before ingestion) (0.777; 95 % CI; -1.55 – 3.10) at week 12 there was no significant change observed in total energy intake between the two groups at any time point.

Table 4.3: Metabolic parameters at baseline and week 12

Parameter	Experimental	Placebo	Difference [a] (95 % CI)
Fasting blood glucose (mmol/L)			
Baseline	5.6±0.6	6.6±1.5	
Week 12	5.6±1.1	6.6±1.7	0.038 (-0.62 – 0.69)
Triglycerides (mmol/L)			
Baseline	2.12±1.4	2.6±1.8	
Week 12	0.6±0.2[b]	0.8±0.3[b]	0.271 (-1.19 – 1.24)
Total cholesterol (mmol/L)			
Baseline	4.3±1.4	4.3±1.4	
Week 12	3.7±0.8	4.0±1.1	0.449 (-1.60 – 0.69)
HDL cholesterol (mmol/L)			
Baseline	0.9±0.2	0.7±0.3	
Week 12	1.0±0.4	0.9±0.6	-0.091 (-0.50 – 0.32)
LDL cholesterol (mmol/L)			
Baseline	3.0±1.3	3.0±1.1	
Week 12	2.5±1.0	2.9±1.3	0.389 (-1.49 – 0.72)
HDL: LDL ratio (mmol/L)			
Baseline	0.4±0.4	0.3±0.1	
Week 12	0.4±0.3	0.3±0.1	0.022 (-0.27 – 0.31)
Leptin ng/mL			
Baseline	25.1±18.3	26.1±16.8	
Week 12	28.8±20.3	24.9±8.8	4.884 (-14.07 – 23.84)

Values are expressed as means ± SD. [a] Treatment minus Placebo. [b] A significant difference was observed at week 12 compared to baseline data ($p < 0.05$). Exclusion of the value zero of the 95 % CI implies statistical significance at the 5 % level.

Table 4.4: Food intake assessment at baseline, week 6 and week 12

Parameter	Experimental	Placebo	Difference [a] (95 % CI)
Carbohydrate intake (g)			
Baseline	226.5±70.3	230.2±75.3	
Week 6	201.8±93.5	193.7±37.3	11.833 (-56.66 – 80.33)
Week 12	183.5±99.6	209.5±92.4	-22.381 (-106.74 – 61.98)
Fat intake (g)			
Baseline	101.8±40	83.8±44.9	
Week 6	66.9±37.6	69.9±21.9	-21.030 (-56.89 – 14.83)
Week 12	54.9±21.5[b]	52.2±22.1[b]	-15.328 (-51.49 – 20.84)
Saturated fat intake (g)			
Baseline	41±15.9	33.6±20.8	
Week 6	26.8±17	26.1±7.8	-6.771 (-22.79 – 9.25)
Week 12	18.4±6.9[b]	18±8.2[b]	-6.976 (-21.74 – 7.79)
Protein intake (g)			
Baseline	98.6±44.8	100.8±31	
Week 6	95.2±47.1	92.4±28.3	4.993 (-34.40 – 44.39)
Week 12	92.9±40.2	84.2±29.1	10.974 (-19.36 – 41.31)
Sodium intake (mg)			
Baseline	3405.8±1679.3	2807.0±1129.1	
Week 6	2518.5±1048	3067.8±3594.6	-1148.143 (-3048.08 – 751.80)
Week 12	2156.2±885[b]	1968.9±913	-411.575 (-1651.21 – 828.06)
Energy intake (kJ)			
Baseline	9105±3473	8805±3330	
Week 6	8386±3431	7438±1522	647.960 (-2096.84 – 3392.76)
Week 12	7552±2527	7484±2385	-231.919 (-2879.56 – 2415.72)

Values are expressed as means ± SD. [a] Treatment minus Placebo. [b] A significant difference was observed at week 12 compared to baseline data ($p < 0.05$). Exclusion of the value zero of the 95 % CI implies statistical significance at the 5 % level.

Table 4.5: Appetite sensations at baseline, week 6 and week 12

Parameter	Experimental	Placebo	Difference [a] (95 % CI)
Before ingestion			
Hunger			
Baseline	4.7±2.5	3.4±2.4	
Week 6	5.6±2.2	5.5±2.8	0.768 (-3.27 – 1.73)
Week 12	6.4±2.2	4.5±2.3[c]	0.777 (-1.55 – 3.10)
Desire to eat			
Baseline	5.2±2.5	3.9±2.6	
Week 6	5.8±2.0	5.1±2.8	0.339 (-2.75 – 2.07)
Week 12	6.2±2.2	4.4±2.4[c]	0.856 (-1.56 – 3.27)
Post ingestion			
Hunger			
Baseline	0.7±1.8	0.4±0.8	
Week 6	2.0±2.8	1.1±1.5	0.750 (-1.47 – 2.97)
Week 12	1.9±2.6	1.9±2.4	0.134 (-2.46 – 2.19)
Desire to eat			
Baseline	1.6±2.0	0.9±1.5	
Week 6	1.9±2.1	0.5±0.9[c]	0.625 (-1.45 – 2.70)
Week 12	1.9±2.5	1.9±2.2	0.344 (-2.52 – 1.83)
Fullness			
Baseline	8.4±1.9	7.3±2.5	
Week 6	8.6±1.5	8.2±2.0[c]	-1.649 (-2.72 – 0.58)
Week 12	8.3±1.9	8.7±1.4[c]	-2.029 (-3.56 – 0.50)
Palatability			
Baseline	6.4±2.8	5.8±3.0	
Week 6	5.9±2.7	6.0±3.2	-1.732 (-3.67 – 0.21)
Week 12	6.1±2.5[b]	6.5±2.8	-1.577 (-3.33 – 0.18)
Meal weight (g)			
Baseline	277.6±101.2	232.6±102.4	
Week 6	261.9±120.4	238.4±70.5	-24.060 (-129.71 – 81.59)
Week 12	248.8±136.9	287.6±95.4	-92.583 (-200.69 – 15.52)

Appetite assessment was performed using the visual analogue scales method (VAS) method. Values are expressed as mean ± SD. [a] Treatment minus Placebo. [b] A significant difference was observed at week 12 compared to baseline data ($p < 0.05$). [c] There was a significant change between groups. Exclusion of the value zero of the 95 % CI implies statistical significance at the 5 % level.

In general, the *C. fimbriata* and placebo capsule preparations in our study were well tolerated. There were few adverse events and these were considered mild. Two participants in the experimental group experienced minor side effects in the first few weeks of the intervention period which included a skin rash and constipation. These symptoms subsided within two weeks following cessation of the intervention.

4.6 Discussion

The present study demonstrated that supplementation with *C. fimbriata* extract in combination with a hypocalorie diet was associated with a clinically significant reduction in central adiposity, a major component of metabolic syndrome. The primary finding was a decline in waist circumference following 12 weeks supplementation in the experimental group. The observed treatment effect (treatment minus placebo) is markedly stronger in this study compared to that reported in the study by Kuriyan et al (2007), with an intervention period of two months.

Obesity is considered a major health problem, increasing the risk of metabolic syndrome (Kuriyan et al., 2007), CVD, type 2 diabetes and other lifestyle related diseases (Cameron et al., 2007). Obesity and its associated co-morbidities continue to present an escalating challenge to contemporary medicine. Waist circumference is a useful and convenient measure of central obesity. Therefore the decline in waist circumference following *C. fimbriata* supplementation is vital as it implicates the potential role of this plant extract in the treatment of central obesity and the prevention of metabolic syndrome and other lifestyle related diseases.

The waist circumference significantly declined independent of body weight in the experimental group after 12 weeks intervention. The relatively greater reduction in waist circumference in the experimental group could indicate higher fat mobilisation induced by *C. fimbriata.* A similar result was reported by Kuriyan et al (2007). This may be due to different rates of lipolysis in different depots of body fat during negative energy balance (Kuriyan et al., 2007). Another possibility may be an increase in lean muscle tissue parallel to fat loss with the increase in energy expenditure. Furthermore it may be attributed to the role of the pregnane glycosides, the main chemical component of *C. fimbriata.* Studies have shown that pregnane glycosides are involved in the inhibition of adipocyte proliferation, differentiation and maturation. A previous study has shown that *C. fimbriata* has the potential to prevent hyperplastic obesity in mice 3T3-L1 pre-adipocyte cell line samples (Akbarsha et al., 2010). It has also been demonstrated that *C. fimbriata* extract is capable of inhibiting adipocyte maturation (Akbarsha et al., 2010). Pregnane glycosides have been reported to inhibit pre-adipocyte cell division in the early phase of adipogenesis by either down-regulation of cyclin-dependent kinase (CDK) or inhibition of import cyclin D1-CDK/6 complex into the nucleus (Akbarsha et al., 2010). Several other studies also found that adipocyte proliferation and differentiation in adipose tissue were inhibited by pregnane glycosides (De Leo et al., 2005, Plaza et al., 2005, Cioffi et al., 2006).

Further research into the underlying mechanisms of *C. fimbriata* extract on central obesity reduction is needed. Measurement of body composition using Dual Energy X-ray Absorptiometry (DXA) and/or three dimensional whole body laser scanning would be able to provide information on muscle mass, segmental body fat content and distribution. In addition animal studies focusing on the expression of genes and enzymes associated with lipogenesis

and lipolysis would be useful in elucidating the particular role of *C. fimbriata* extract in central obesity reduction.

A waist circumference indicative of central obesity is linked with a chronic inflammatory state, promoted by low-grade plasma increases in the adipocytokines including circulating leptin, TNF-α, PAI-1 and a reduction in adiponectin (Carr et al., 2004). *C. fimbriata* extract has been shown to significantly reduce leptin levels and inhibit leptin resistance in rat studies (Kamalakkannan et al., 2010). However, the present study did not show a significant reduction in leptin levels following 12-week *C. fimbriata* administration. Further human trials of a longer intervention period investigating the effect of *C. fimbriata* supplementation on adipocytokines are warranted considering the promising results reported in rat models.

In our study, WHR was significantly reduced following supplementation of the *C. fimbriata* extract with a balanced dietary intake and physical activity level in the experimental group. Previous studies have shown that a smaller WHR is associated with a reduced risk of developing impaired glucose metabolism, type 2 diabetes, CVD (Rocha et al., 2008) and also an improvement in blood lipid profile (Seidell et al., 2001). In addition a smaller WHR is associated with a lower risk of metabolic syndrome disturbances, including lower triglyceride and glucose levels, increased HDL-cholesterol (Snijder et al., 2004a, Snijder et al., 2004b) and lower BP (Snijder et al., 2004a).

The improvement in triglyceride levels and systolic BP, and the reduction in anthropometric parameters including, body weight and BMI in both experimental and placebo groups in the present study could possibly be attributed to a combination of controlled dietary intake, healthy dietary choices and exercise. Previous studies have shown that participation in

structured exercise programs reduces the prevalence of metabolic syndrome (Santos et al., 2007). Moderate exercise is beneficial in modifying components of metabolic syndrome, including promoting loss of central fat accumulation, increasing muscle mass (Santos et al., 2007), improved insulin sensitivity (Pratley et al., 2000), reduced BP (Stewart, 2002), increased HDL cholesterol and lower triglyceride levels (Lutsey et al., 2008, Yuliana et al., 2011, Whelton, 1996). It should be noted that all data on physical activity in the current study were based on self-reported information. Lack of monitoring on this was considered a limitation.

Both placebo and experimental groups showed a significant reduction in the intake of total fat and saturated fat after 12 weeks intervention. In addition we also observed an increasing trend in the consumption of whole grains, fruit and vegetables. Healthy dietary patterns high in whole grains, fruit, vegetables and low in saturated fat are fundamental recommendations for metabolic syndrome (Lutsey et al., 2008). The increase in the consumption of whole grains in this study may have contributed to the significant reduction in systolic BP, BMI and triglyceride levels in both groups (Lutsey et al., 2008).

Whole grain, fruit and vegetable intake is linked with a decline in hunger and an increase in satiation, which may in turn cause a voluntary reduction in energy intake (Kuriyan et al., 2007). The human trial investigating the effect of *C. fimbriata* extract on obese Indians observed a significant decline in hunger, energy intake and less desirable foods including refined sugars, saturated fat, cholesterol and sweets after two months intervention (Kuriyan et al., 2007). In the present study no significant change in energy intake between the two groups was recorded over the intervention period although the placebo group showed a reduction in hunger and desire to eat at week 12. For the experimental group no marked change in all

elements of appetite sensations except palatability was observed. The reduction in the consumption of less desirable foods indicates that the reward circuitry in the brain may be interrupted by supplementation, therefore affecting feeding behaviour (Yuliana et al., 2011). This has been reported in other studies with medicinal plant supplementation, such as the alkaloid-rich *Mitragyna speciosa* (Thongpradichote et al., 1998). More investigations are needed to elucidate the underlying mechanisms of pregnane glycosides on feeding behaviour and appetite regulation.

In the current study sodium intake in the experimental group was reduced after 12 weeks intervention. The significant reduction in the palatability of the test meal in this group could be associated with the decline in sodium intake. There may have been a change in taste sensation in the experimental group, which possibly led to a decreasing trend in food consumption and a reduction in the desire of consuming salty food in the experimental group. It is well known that sodium intake is strongly linked to BP regulation with reductions in sodium intake being strongly associated with reductions in systolic BP (Grundy et al., 2005). Clinical trials have demonstrated that reduced salt intake lowers BP in participants at risk of metabolic syndrome (Whelton, 1996). The significant reduction in sodium intake and palatability of meal in the experimental group could have possibly contributed to the reduction in systolic BP. However, more investigations are needed to elucidate these effects of *C. fimbriata* extract on taste sensation, salt intake and BP.

Further research into the underlying mechanisms of *C. fimbriata* extract on central obesity reduction is needed. In addition to what has been proposed in the previous paragraphs, further investigations on the effects of this supplement on inflammatory biomarkers in obese adults will also add to the understanding of the mechanisms behind the reduction in intra-abdominal

fat mass. Moreover, double blind randomised controlled clinical trials of a longer duration and of a larger sample size would also be useful to understand the efficacy of *C. fimbriata* extract on the long-term treatment of obesity and associated lifestyle related diseases.

4.7 Conclusion

The present study suggests that supplementation with *C. fimbriata* extract was associated with a clinically meaningful reduction in central adiposity. The controlled exercise recommendations and modifications to dietary intake were linked with favourable changes of metabolic risk factors and an improvement in general health and wellbeing in overweight and obese Australian adults. This study may hold therapeutic promise as an approach for the treatment of obesity and associated lifestyle related risk factors.

4.8 Study specific acknowledgements

We wish to thank the study participants for their cooperation and effort. The raw *Caralluma fimbriata* powder used in this study was provided by Gencor Pacific Group, Hong Kong. Both *C. fimbriata* and placebo capsules were formulated by AZPA Pty Ltd, Melbourne, Australia. We thank the late Mrs Dawn Bannon for her help with blood collection. We also like to thank Dr Crispin Dass for his assistance with the FLUO star/POLAR Galaxy microplate reader. We wish to thank Dr Ewa Sztendur and Dr Mark Scarr for their assistance with statistical analysis. We greatly appreciate the assistance from the nutritional therapy students and staff at the Victoria University Nutritional Therapy Clinic with nutrition consultations. Furthermore, we wish to thank the technical staff of the School of Biomedical and Health Science for their support with the test breakfast.

CHAPTER 5: STUDY 2 - METABOLIC EFFECTS OF CHRONIC ADMINISTRATION OF CARALLUMA FIMBRIATA EXTRACT IN RATS WITH DIET INDUCED OBESITY

5.1 Abstract

Objective: The prevalence of overweight and obesity has reached epidemic proportions worldwide. Prevention and management strategies of this common nutrition disorder are limited. Therefore the search for natural alternative therapies has become increasingly popular. However, the efficacy of many botanical extracts in the prevention and management of obesity is yet to be fully explored. Our previous human trial found that *C. fimbriata* extract is capable of reducing waist circumference in overweight and obese adults, therefore it would be of interest to further investigate the functional role of *C. fimbriata* administration on metabolic comorbidities in obese rodents. The aim of this study was to investigate the metabolic adaptations which occur in response to chronic administration of *C. fimbriata* extract in an animal model of diet induced obesity.

Design: Male Wistar rats were assigned into four groups: Standard chow, high fat, standard chow + *C. fimbriata* or high fat + *C. fimbriata*. Following seven weeks of high fat diet to induce obesity, male Wistar rats were orally administered with 100 mg/kg BW (n = 10) or placebo (n = 10) for eight weeks while being maintained on either the high fat diet or standard chow diet.

Results: There was no significant overall treatment effects observed in the *C. fimbriata* treated groups for food intake ($p = 0.14$), body composition ($p > 0.05$), systolic and diastolic blood pressure ($p = 0.48$, $p = 0.43$ respectively), lipid profile and insulin sensitivity and glucose tolerance ($p > 0.05$).

Conclusion: This study revealed that treatment with *C. fimbriata* extract in lean and obese rats does not have an effect on cardio-metabolic associated abnormalities.

5.2 Introduction

Excess visceral fat deposition in the abdominal cavity is a major risk factor for CVD, type 2 diabetes and metabolic syndrome (Despres, 2007, Ibrahim, 2010). Obesity is a very common nutrition disorder and has become a global burden on the health care system, with the prevalence of overweight and obesity steadily increasing for the past 30 years. At present, the use of prescription drugs has modest clinical efficacy for obesity treatment, however these anti-obesity drugs are often associated with gastrointestinal, cardiovascular and central nervous system adverse effects (Pagotto et al., 2008). Therefore the quest for natural products that are safe and effective in aiding weight loss has been intensified.

Owing to the adverse side effects of many anti-obesity drugs, more recent trials have focused on natural botanical sources that have been reported to reduce appetite and body composition parameters with minimal side effects. Many botanical extracts have been used for centuries in folk medicine to reduce obesity (Hasani-Ranjbar et al., 2013). However, the clinical efficacy and mechanism of action of many botanicals have not been well explored. Dietary strategies for the treatment of obesity have been mostly ineffective due to in part the feelings of hunger which undermine adherence to weight loss regimens (Mattes and Bormann, 2000). The identification of botanical extracts that promote satiety or that at least sustain satiety during energy restriction is highly in demand.

C. fimbriata is a commonly known edible succulent plant native to India. Since the Vedic period, *C. fimbriata* has been used by Indian tribal people as an appetite suppressant (Dutt et

al., 2012). The hydroethanolic extract of this perennial plant is currently sold over the counter as a botanical supplement with potential anorectic properties. As shown in the previous chapter, our first human trial demonstrated the anti-obesity properties of *C. fimbriata* extract administration in overweight and obese adults (Astell et al., 2013a). Following this finding, it was evident that further investigations were required to better clarify the functional role of *C. fimbriata* in eliciting protective metabolic effects in the obese state. In addition, it was observed in a previous study conducted by Kamalakkannan et al (2010) that *C. fimbriata* administration in rodents reduced appetite and prevented gains in body weight, liver weight and fat pads, as well as inhibited alterations in blood lipid profile, leptin levels and prevented the development of atherosclerosis. The administration of *C. fimbriata* extract was designed as a preventive therapy in rats fed a cafeteria diet. Rats were administered *C. fimbriata* by gavage from day one at three different doses (25, 50 & 100 mg/kg BW) for 90 days. There have not been any animal studies to our knowledge that have investigated the effects of *C. fimbriata* extract on the treatment of all components of metabolic syndrome after inducing obesity, and with the promising anti-obesity properties observed in our previous human trial, the present study was undertaken to determine the effectiveness of *C. fimbriata* extract on the treatment of metabolic syndrome in obesity induced rats, by investigating its effects on organ weight, liver histology, body composition, appetite, cholesterol and triglyceride levels, diabetes risk and hypertension.

5.3 Materials and Methods

5.3.1 Animals and housing

The Animal Ethics Committee of the Howard Florey Institute, University of Melbourne approved the experimental protocol (AEC 11-037). The rats were housed in individual cages

and were provided with *ad libitum* of tap water and the group-specific rat diet. The animals were housed in an environmentally controlled laboratory with a seven day acclimatization period prior to commencement of the trial. The animal facility was maintained under stable conditions with a 12 hour light – 12 hour dark cycle (lights off at 19.00 hours), a constant temperature of 22 °C ± 2 °C and relative humidity of approximately 60 %.

5.3.2 Experimental design

Male Wistar rats (4 weeks old) were divided into four groups, with 10 rats in each group (n = 40). For randomisation, rats were allocated into four groups based on their body weight and usual food intake. The study was over a 15 week period: the initial seven weeks to induce obesity and the following eight weeks for *C. fimbriata* extract treatment. For the first seven weeks, rats were fed either the standard pellet chow diet (n = 20) or high fat diet (n = 20). Please refer to Table 3.7 (3.5.1 Body weight and food consumption) for the macronutrient breakdown of the standard chow and high fat diets. It was expected by seven weeks that rats fed the high fat diet would present with obesity (Buettner et al., 2007). For the following eight weeks, rats received no supplement or a daily dose of *C. fimbriata* extract of 100 mg/kg BW per day (powder form mixed with a peanut butter vehicle). A previous study also used this dose (Kamalakkannan et al., 2010). The two groups receiving no supplement i.e. only standard chow (n = 10) or high fat (n = 10) diets were given the peanut butter only.

5.3.3 Metabolic measurements

Food intake and body weight were recorded daily throughout the study (Refer to section 3.5.1). Abdominal circumference (AC) was assessed post-intervention in anaesthetized rats

(section 3.5.2). Whole body composition (fat mass, lean mass, free water and total body water) was determined by using the EchoMRI-900 body composition analyser at baseline, midway and at the end of the supplementation period (section 3.5.2). Blood pressure (section 3.5.3), 24-hour urine collection (section 3.5.4), sodium excretion (section 3.5.5), insulin sensitivity (section 3.5.6) and glucose tolerance (section 3.5.7) were measured at baseline and at the end of the supplementation period. At time of death, rats were anaesthetised and abdominal adipose tissue and liver was dissected and weighed. Rats were euthanized following cardiac puncture. A 10 mL blood sample was collected for the analysis of plasma glucose, triglycerides and total cholesterol (as outlined in section 3.6).

5.3.4 Histopathological examination

Hepatic tissue samples were fixed in 10 % neutral-buffered formalin (NBF) and embedded in paraffin and standard serial sections were cut using a microtome. Hepatic tissue samples were stained with haematoxylin and eosin according to a standard protocol (Ross and Pawlina, 2011) and examined with an optical microscopy (As outlined in section 3.8.2).

5.3.5 Liver lipid content

Three grams of hepatic tissue was cut finely and put into bottles containing 50 mL of chloroform: methanol (2:1) and approximately 10 mg/L butylated hydroxytoluene and were kept in the cool room overnight for lipid extraction. Lipid extraction was performed according to the Folch procedure (Folch et al., 1957). The solvent containing lipids was evaporated under vacuum in a rotary evaporation (Heidolph VV 2000, waterbath type WB

2000, Schwabach, Germany). The lipid content was determined gravimetrically (Su et al., 2004) (as outlined in section 3.8.1).

5.4 Statistical analysis

All data were expressed as mean ± SEM and statistical analysis was performed using the SPSS package, version 21 (SPSS, Chicago, IL, USA). One-way ANOVA was used to measure significant differences between groups at one time point for plasma analysis, liver lipid content, organ weight and abdominal circumference and where the main effect was significant, means were compared with Tukeys Post Hoc test. For all other data, mixed model ANOVA was used to analyse the effects of the intervention, time, and the interaction between the intervention and time with pairwise comparisons (adjusted for multiple comparisons by Bonferroni's post-hoc test). When the interaction and/or the main effects were significant, means were compared using Tukey's multiple comparison post hoc test. Statistical significance was set as $p < 0.05$.

5.5 Results

Feeding behavior was monitored on a daily basis in the test animals over the intervention period. There was a significant time effect observed in average food intake from baseline (week 7) to post-intervention (week 15) ($p = 0.03$). However, there was no significant interaction for food intake observed ($p = 0.14$) (Figure 5.1).

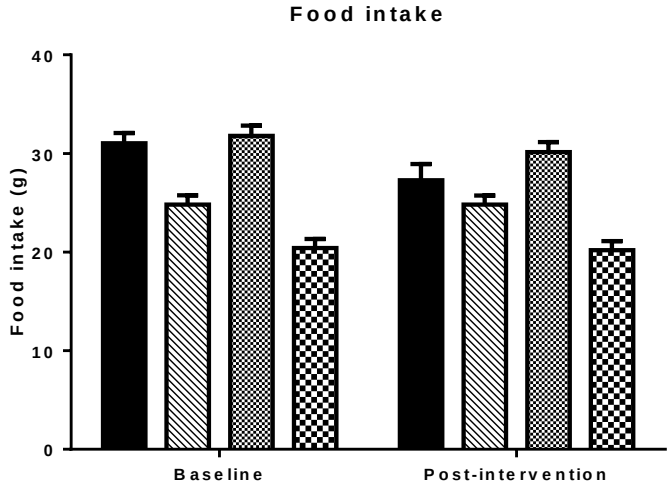


Figure 5.1: The effect of *C. fimbriata* extract on food intake. STD CHOW , standard chow; HF , high fat; STD + CFE , standard chow plus *C. fimbriata* extract; HF + CFE , high fat + *C. fimbriata* extract. Values are means (n 10), with their standard errors represented by vertical bars. Statistical analysis was performed using mixed model ANOVA.

Post-intervention a blood sample was collected for the analysis of plasma total cholesterol, plasma triglycerides, and plasma glucose. There was a significant difference between groups for post-intervention total cholesterol levels ($p = 0.004$) and triglycerides ($p = 0.001$) (Table 5.1). There was no significant difference between groups for plasma glucose ($p = 0.07$). The STD CHOW + CFE had a significantly lower total cholesterol level compared to all other groups ($p<0.05$). Both standard chow groups had a significantly lower triglyceride level and liver lipid content compared to both high fat diet groups ($p <0.05$).

Table 5.1: Post-intervention plasma analysis and liver lipid content

Variable	*Standard chow*	*High fat*	*Standard chow + C. fimbriata*	*High fat + C. fimbriata*	P value
Plasma glucose (mmol/L)	11.55 ± 0.48	14.93 ± 2.10	12.05 ± 0.94	16.26 ± 1.56	0.07
Total cholesterol (mmol/L)	1.52 ± 0.07[b]	1.54 ± 0.10[b]	1.05 ± 0.07[a]	1.58 ± 0.11[b]	0.0004
Triglycerides (mmol/L)	1.61 ± 0.18[b]	2.75 ± 0.42[a]	1.47 ± 0.12[b]	2.59 ± 0.22[a]	0.001
Liver lipid content (g)	5.16 ± 0.29[b]	6.99 ± 0.50[a]	5.21 ± 0.19[b]	6.43 ± 0.48[a]	0.008

Values are means (n 10), with their standard errors. Statistical analysis was performed using one-way ANOVA.

[a,b] Mean values within a row with unlike superscript letters were significantly different ($P<0.05$).

Pre-intervention and post-intervention blood pressure was monitored via the tail cuff method. There were no significant differences between groups for systolic and diastolic blood pressure ($p >0.05$) (Table 5.2). Urine samples were also collected to measure urine volume. There was a significant time effect for urine volume, with a mean increase of 6.8 mL ($p = 0.01$). However, there was no significant difference between groups for urine volume ($p = 0.45$) or plasma sodium excretion ($p = 0.67$).

Table 5.2 Blood pressure recordings, urine volume and sodium excretion

Variable	Baseline	Week 15	Baseline	Week 15	Baseline	Week 15	Baseline	Week 15	P value	
	Standard chow		*High fat*		*Standard chow + C. fimbriata*		*High fat + C. fimbriata*			
Systolic BP (mmHg)	134.38 ± 7.33	124.0 ± 7.14	123.25 ± 6.36	127.84 ± 3.97	135.58 ± 7.51	132.13 ± 5.21	132.62 ± 3.28	123.42 ± 5.76	Group Time Interaction	0.70 0.33 0.48
Diastolic BP (mmHg)	100.26 ± 5.76	89.45 ± 11.66	89.94 ± 5.99	92.67 ± 3.70	89.93 ± 4.14	94.44 ± 7.46	97.04 ± 3.96	86.22 ± 4.40	Group Time Interaction	0.95 0.44 0.43
Urine volume (mL)	12.10 ± 1.45	16.76 ± 3.0	11.47 ± 4.54	23.91 ± 4.79	11.31 ± 1.26	13.93 ± 2.65	11.75 ± 2.85	20.23 ± 4.53	Group Time Interaction	0.57 0.01 0.45
Sodium Excretion (mmol/L)	56.37 ± 7.27	65.76 ± 6.19	62.18 ± 11.52	82.19 ± 4.54	64.59 ± 6.80	64.01 ± 13.61	68.52 ± 4.56	84.03 ± 9.11	Group Time Interaction	0.34 0.09 0.67

Values are means (n 10), with their standard errors. Statistical analysis was performed using mixed model ANOVA.

The effect of *C. fimbriata* extract on body composition of rodents including abdominal circumference, body weight, total body fat %, total body lean mass and organ weight was measured (Figure 5.2 & 5.3). There was a significant difference between groups for liver weight ($p = 0.01$) and kidney weight ($p = 0.008$). Both STD CHOW groups had a significantly smaller liver compared to the HF + CFE group ($p < 0.05$). Also, both STD CHOW groups had a significantly larger kidney compared to the HF + CFE group ($p <0.05$). There was a significant difference between groups for perirenal fat ($p = 0.003$) and epididymal fat ($p = 0.05$). Both high fat groups had a significantly greater amount of perirenal and epididymal fat compared to the STD CHOW groups ($p <0.05$).

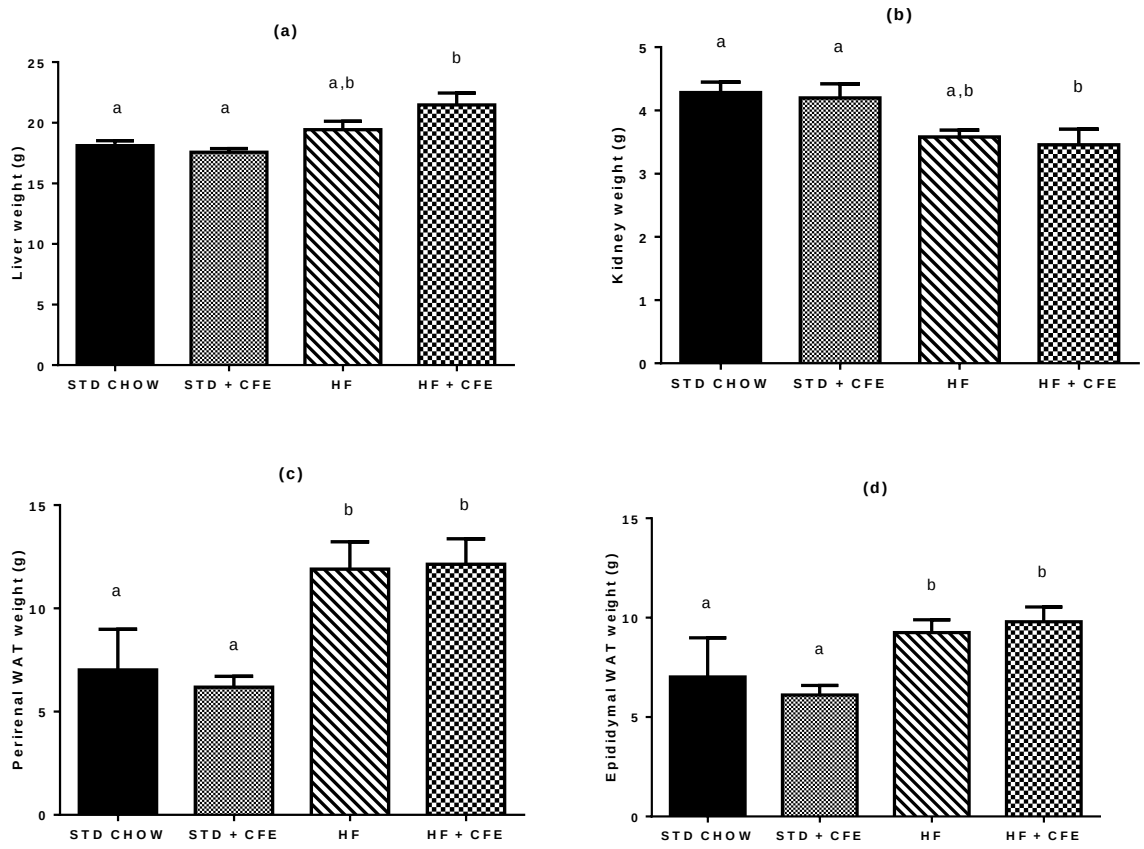


Figure 5.2 a - d: The effect of *C. fimbriata* extract on organ weight and fat pad mass. STD CHOW , standard chow; HF , high fat; STD + CFE , standard chow plus *C. fimbriata* extract; HF + CFE , high fat + *C. fimbriata* extract; WAT, white adipose tissue. Values are means (n 10), with their standard

errors represented by vertical bars. Statistical analysis was performed using one-way ANOVA. [a,b] Mean values within a column with unlike superscript letters were significantly different (P<0.05).

There was a significant difference observed between groups for abdominal circumference (p = 0.02) (Figure 5.3 a). The STD CHOW + CFE had a significantly smaller abdominal circumference compared to the high fat group (p = 0.01). There was a significant time effect for body weight (mean gain of 126.52 g) (p = 0.0001), total body fat percentage (Mean increase of 5.66 %) (p = 0.0001) and total body lean mass (Mean decrease of 4.52 %) (p = 0.0001). However, there were no significant interactions (time* group) observed for body weight (p = 0.07), total body fat percentage (p = 0.32) and total body lean mass (p = 0.30).

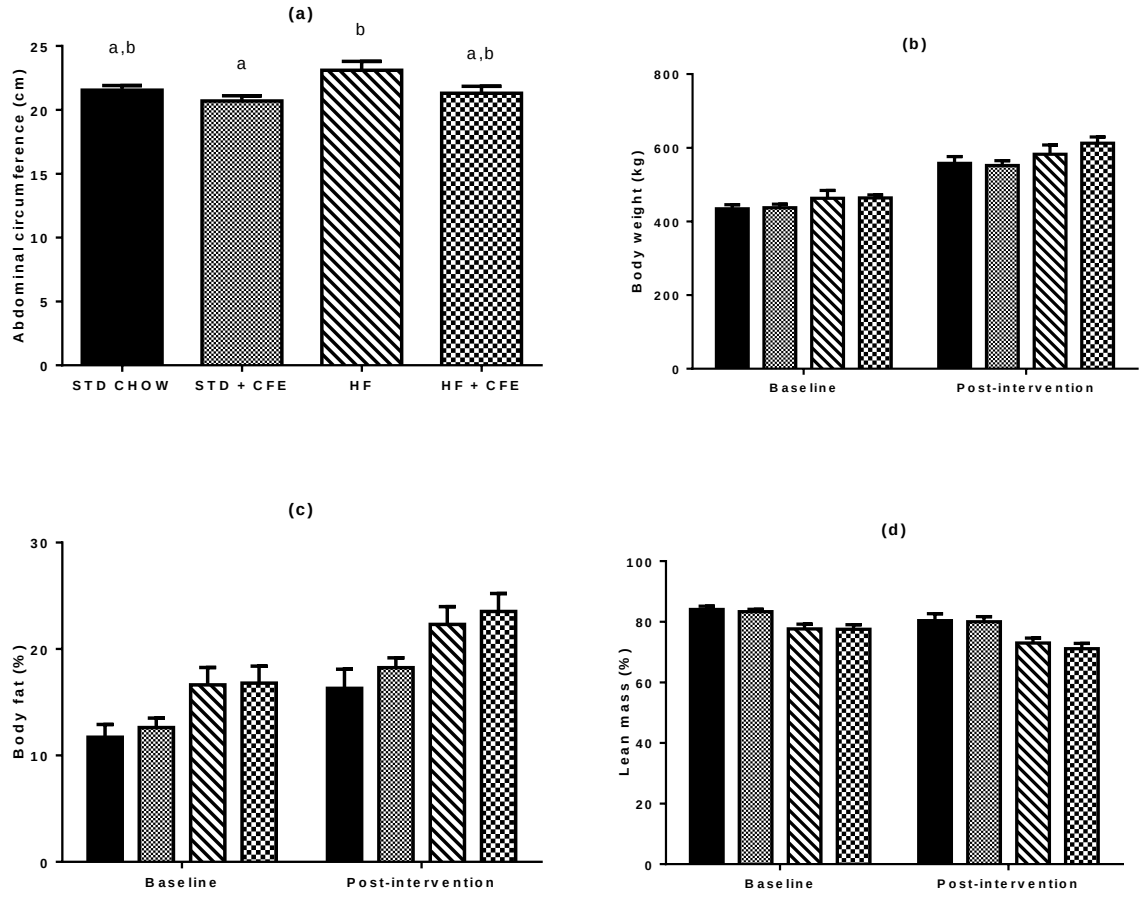


Figure 5.3 a - d: The effect of *C. fimbriata* on body composition. STD CHOW , standard chow; HF , high fat; STD + CFE , standard chow plus *C. fimbriata* extract; HF + CFE , high fat + *C.*

fimbriata extract. Values are means (n 10), with their standard errors represented by vertical bars. Statistical analysis was performed using one-way ANOVA for abdominal circumference & mixed model ANOVA for body weight, body fat & lean mass. [a,b] Mean values within a column with unlike superscript letters were significantly different (P<0.05).

At baseline and post intervention glucose tolerance and insulin sensitivity were measured and expressed as area under the curve (AUC) (Figure 5.4 & 5.5). A time by group interaction was observed for glucose AUC (p = 0.01). The HF group had a significantly higher glucose AUC than both STD CHOW groups (p = 0.001) and the HF + CFE tended to have an improved glucose metabolism compared to the HF group (p = 0.08). While for the insulin sensitivity AUC, there was only a significant time effect observed (p = 0.001) (Figure 5.5).

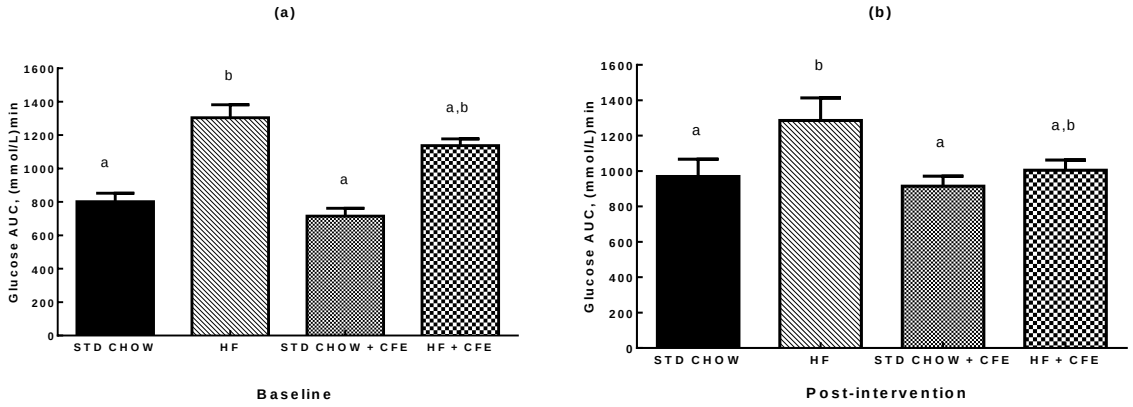


Figure 5.4 a - b: The effect of *C. fimbriata* on glucose metabolism AUC. STD CHOW , standard chow; HF , high fat; STD + CFE , standard chow plus *C. fimbriata* extract; HF + CFE , high fat + *C. fimbriata* extract. Values are means (n 10), with their standard errors represented by vertical bars. Columns with different letters indicate significant differences (p <0.05). Statistical analysis was performed using mixed model ANOVA.

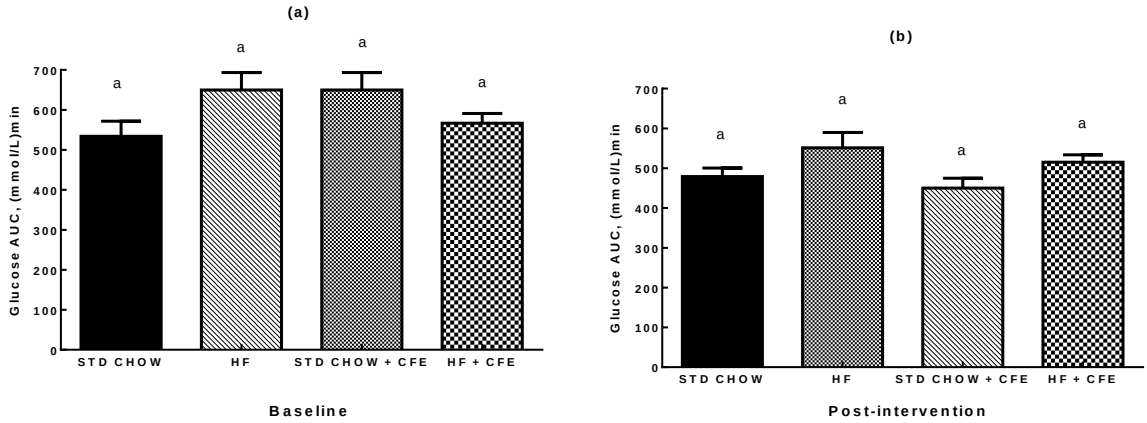


Figure 5.5 a - b: The effect of *C. fimbriata* on insulin sensitivity AUC. STD CHOW ■, standard chow; HF ▨, high fat; STD + CFE ▩, standard chow plus *C. fimbriata* extract; HF + CFE ▩, high fat + *C. fimbriata* extract. Values are means (*n* 10), with their standard errors represented by vertical bars. Columns with different letters indicate significant differences ($p < 0.05$). Statistical analysis was performed using mixed model ANOVA.

The effect of *C. fimbriata* extract on hepatic histology was assessed at post-intervention. Histological examination of the H & E stained liver showed increased hepatic lipid deposition in the high fat groups (Figure 5.6 C, D) compared to the STD CHOW groups (Figure 5.6 A, B). The histological examination of the H & E stained STD CHOW and STD + CFE liver sections showed normal architecture and hepatocytes (Figure 5.6 A, B).

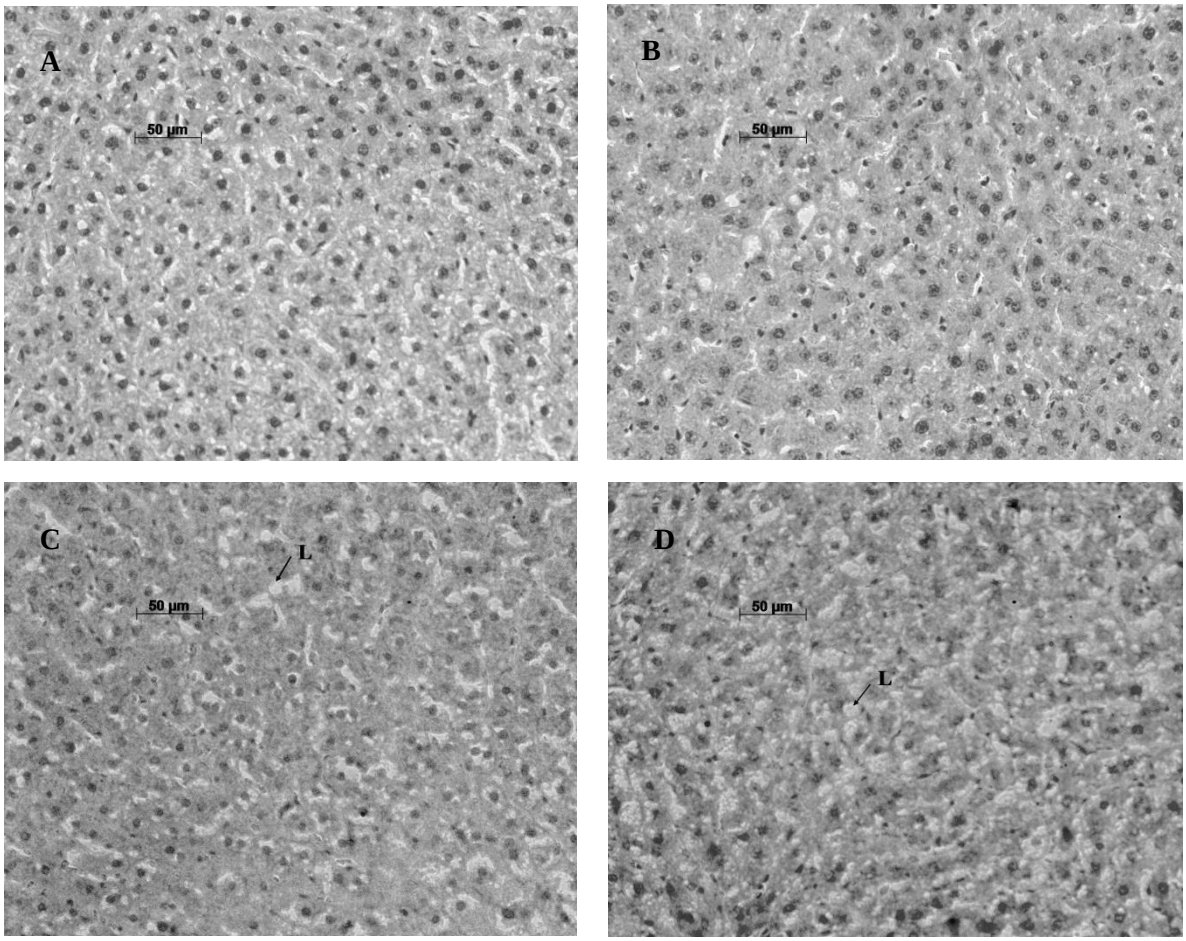


Figure 5.6: Histological analysis of liver tissue stained with hematoxylin and eosin (x400) showing the various degrees of lipid droplet formation in all of the four groups. Representative microphotographs of H & E stained sections of liver tissue from STD CHOW; standard chow (A), STD CHOW + CFE; standard chow + *C. fimbriata* (B) and HF + CFE; High fat + *C. fimbriata* (C). HF; high fat (D), arrows pointing to lipid droplets marked as (L).

5.6 Discussion:

The regulation of food intake is achieved via complex mechanisms which aim to maintain body weight relatively constant over long periods. Appetite regulates the body's desire for food through a complex biological process designed to satisfy the body's need for energy and macronutrients (Beckman et al., 2005). Therefore appetite plays an imperative role in the

regulation of body weight control, and notably obese individuals have been shown to have increased appetite and eating disorders (Spitzer et al., 1993). Thus measures to reduce hunger and increase satiation in overweight and obese individuals through appetite regulation could help in preventing further weight gain and enhance weight regulation.

It was hypothesised that *C. fimbriata* extract would reduce appetite, thereby decreasing feed intake. However, the current study did not observe a treatment effect on food intake, which was also the case in our human trial (Astell et al., 2013a). This result was also evident in a study evaluating the safety of *C. fimbriata* extract in rats (Odendaal et al., 2013a). Odendaal et al (2013) measured the treatment related toxicity of *C. fimbriata* extract over a six month period at three doses of 100, 300 & 1000 mg/kg BW. Interestingly, there was no effect on food intake or body weight during the intervention period (Odendaal et al., 2013a). The current literature on the use of *C. fimbriata* as an effective appetite suppressant is inconsistent. Contrary to the findings reported in this study, previous animal trials that have found a significant effect of *C. fimbriata* administration on hunger levels and food intake (Komarnytsky et al., 2013, Kamalakkannan et al., 2010). It is thought that *C. fimbriata* extract may reduce food intake through multiple mechanisms. Appetite control as reflected in the decline in food intake may occur at the hypothalamic level, where the key phytochemical ingredient, pregnane glycosides found in *C. fimbriata* are known to act (MacLean and Luo, 2004). The mechanism behind the reduction in food intake through the action of pregnane glycosides was recently investigated by Komarnytsky et al (2013). The authors found that following administration of an *Asclepias incarnata* L. extract (which is rich in pregnane glycosides) at 25-100 mg/kg BW daily, there was a significant reduction in food intake and body weight gain in rodents which was linked with an increase in gastric accommodation and delay of gastric emptying (Komarnytsky et al., 2013). The proximal stomach produces low-

frequency, continuous contractions that create a pressure gradient from the stomach to the small intestine and is therefore accountable for gastric emptying. This propagation of gastric motility comes about from smooth muscle cells assimilating multiple hormonal, chemical and neural signals, the latter signals originating mainly from the enteric nervous system (Cummings and Overduin, 2007). A comparable set of indicators involving the delayed liquid gastric emptying, relaxation of the proximal stomach, and faster small intestinal transit has been reported before in individuals for serotonin receptor agonist MKC-733 (Coleman et al., 2003) and metformin (Cubeddu et al., 2000). Specifically in obese individuals, the speed of gastric emptying is vital in food intake regulation and glucose homeostasis, (Tosetti et al., 1996). Accordingly, reducing the speed of gastric emptying decreases food intake via prolongation of a vagal nerve-mediated satiety reflex (Berthoud, 2008). However, the precise mechanism of action on how pregnane glycosides affect gastric emptying needs further investigation (Komarnytsky et al., 2013).

Komarnytsky et al (2013) also revealed that the effects of treatment with ikemagenin, the major milkweed pregnane glycoside is also promptly transmitted to central tissues namely the hypothalamus, in which the expression of orexigenic and anorexic neuropeptides become affected. Ikemagenin triggered a decline in hypothalamic agouti-related protein (AgRP) mRNA levels (0.6-fold) and caused a rise in the brain-derived neurotropic factor (BDNF) mRNA levels without causing an effect on pro-opiomelanocortin (POMC) and cocaine and amphetamine-regulated transcript (CART) mRNA. Hypothalamic BDNF is a neuronal survival, differentiation and plasticity factor known to serve as a vital constituent of central neural circuits required for the regulation of energy homeostasis. On the other hand, AgRP is a fundamental part of the melanocortin system involved in energy balance and food intake regulation and has antagonistic effects on melanocortin three and four receptors that trigger

an ongoing increase in food consumption (Cone et al., 2001). Collectively, the major findings reported by Komarnytsky et al (2013) support the multimodal effects of pregnane glycosides on food intake regulation which may be mediated by hypothalamic neuropeptides (Komarnytsky et al., 2013), in addition to effects on gastric motility.

There are also other theories put forward as to how the pregnane glycosides reduce appetite. It is thought that the *C. fimbriata* extract may down-regulate ghrelin synthesis in the stomach and neuropeptide-Y in the hypothalamus, resulting in appetite suppression (Shibasaki et al., 1993, Walter et al., 1994, Hulsey et al., 1995, Gardiner et al., 2005). A treatment effect of reducing food intake has also been observed in human studies (Kuriyan et al., 2007). Kuriyan et al (2007) reported that following supplementation with *C. fimbriata* extract (1g/day) in overweight human participants for 2 months, there was a significant reduction in hunger levels and energy intake compared to placebo (Kuriyan et al., 2007). Although in this study, there was no significant difference between groups for body weight, BMI, hip circumference, and percentage body fat which is consistent with our findings of no treatment effect in body weight or percentage body fat. In contrast, other animal studies have observed a significant reduction in body weight gain in obese rodents treated with *C. fimbriata* extract (Kamalakkannan et al., 2010, Ambadasu et al., 2013b, Ambadasu et al., 2013a, Sudhakara et al., 2014). The prevention in fat accumulation observed in animal studies is thought to involve several mechanisms. The pregnane glycosides are believed to elicit anorectic properties by blocking the activity of citrate lyase enzyme, resulting in the inhibition of fatty acid biosynthesis. Moreover, it also blocks the formation of malonyl-coenzyme A, thus enhancing stored fatty acid oxidation, which is similar to the mechanism of *Garcinia Cambogia* (Soni et al., 2004).

Another mechanism behind the prevention of hyperplasic obesity observed in previous studies is through the inhibition of adipocyte proliferation and differentiation in adipose tissue by the pregnane glycosides (De Leo et al., 2005, Plaza et al., 2005, Cioffi et al., 2006). However, due to the inconsistencies in current trials as to whether *C. fimbriata* is capable of reducing body weight as well as suppressing appetite for body weight maintenance, future studies are required to confirm the anorectic properties of *C. fimbriata* extract. Investigations could focus on establishing a higher dose or a range of doses required to achieve a significant change in both body weight and appetite, in addition to investigating the underlying synergistic mechanisms proposed to cause these beneficial effects.

It has been demonstrated in previous studies that *C. fimbriata* extract is capable of reducing waist circumference independent of body weight in randomised controlled human clinical trials (Kuriyan et al., 2007, Astell et al., 2013a). The authors stated that it was unclear as to why the waist circumference specifically declined independent of body weight. It was hypothesised that fat in different regions of the body have different rates of lipolysis during negative energy balance or fasting (Monzon et al., 2002). For example, under lipolytic stimuli with noradrenaline, subcutaneous fat in the anterior abdominal wall has different rates of lipolysis when compared to whole body lipolytic rates (Kurpad et al., 1994). In the present study, there was no significant treatment effect on abdominal circumference or adipose tissue, including perirenal and epididymal fat pads. However, this finding is inconsistent with the previous animal study by Kamalakkannane et al (2010). Morphometric analyses of fat pads revealed a significant decline in perirenal, epididymal and mesenteric fat pads in the *C. fimbriata* extract treated rats (Kamalakkannan et al., 2010). Excess central adiposity around the waist line (deep subcutaneous and visceral adiposity) in overweight or obese individuals is associated with an increased risk of cardio-metabolic disease (Pouliot et al., 1994, Kissebah

et al., 1982) and is a major component used in the diagnosis of metabolic syndrome (Cameron et al., 2007). It would have been useful to have collected other visceral adipose tissue such as omental adipose tissue or subcutaneous adipose weights in both studies to further assess the reported antiobesogenic properties of *C. fimbriata* extract.

There is a close relationship between central obesity, metabolic syndrome and NAFLD, with the majority of NAFLD patients presenting with many metabolic syndrome components (Marchesini et al., 2001). It has been reported in a 90 day oral toxicity study that administration of *C. fimbriata* extract did not show any evidence of treatment related hepatic abnormalities (Odendaal et al., 2013a), which was also the case in the current study. A previous study conducted by Latha et al (2014) found that following administration of *C. fimbriata* extract in diabetic induced rats that there was significant recovery of liver destruction compared to the control group. Latha et al (2014) also observed a reduction in Alkaline phosphatase (ALP) in the *C. fimbriata* treated group, which emphasises the hepatoprotective effects of *C. fimbriata* through its stability of biliary function against the damage caused by streptatozocin induced diabetes.

Furthermore, in support of these findings, administration of *Caralluma edulis* in diabetic rodents has been found to significantly reduce lipid peroxidase in hepatic tissue as compared to diabetic control rats (Sayantan and Abhishek, 2012). Insulin resistance increases the activity of the enzymes which initiate oxidation of fatty acids which results in lipid peroxidation (Sayantan and Abhishek, 2012). Increased lipid peroxidation impairs membrane functions by decreasing membrane fluidity that leads to cell injury and damage (Sayantan and Abhishek, 2012). The lipid peroxidation scavenging activity observed in *Caralluma edulis*

treated rats may be associated with the hepatic protective action observed in *C. fimbriata* extract treated rats.

In addition to the histological observations in the liver following *C. fimbriata* treatment, our study did not find any treatment effect for liver lipid content or total cholesterol and triglycerides. Human clinical trials investigating the efficacy of *C. fimbriata* also failed to demonstrate lipid lowering effects following supplementation of *C. fimbriata* in overweight and obese adults compared to control (Kuriyan et al., 2007, Astell et al., 2013a). Differing results have been observed in previous animal studies which showed a significant improvement in lipid profile in cafeteria fed rats treated with *C. fimbriata* extract (Kamalakkannan et al., 2010, Ambadasu et al., 2013a, Ambadasu et al., 2013b, Somnath et al., 2012, Jagtap et al., 2013, Latha et al., 2014). Due to the conflicting findings observed in human and animal studies, further investigations into the effect of *C. fimbriata* extract on hyperlipidemia is warranted.

Hyperlipidemia is a well-recognized consequence of type 2 diabetes (Wadood et al., 1989). Diabetes induced hyperlipidemia is associated with an accumulated mobilisation of fat in adipose tissue due to underutilization of glucose (Momo et al., 2006). Studies have demonstrated that in diabetic rodents, the rise in blood glucose levels is accompanied by elevated total cholesterol, triglycerides, LDL-cholesterol and a reduction in HDL-cholesterol. Oral administration of *C. fimbriata* extract has been shown to normalize the rise in blood glucose as well as an improvement in lipid profile in other animal studies (Sudhakara et al., 2014, Jagtap et al., 2013). It has been previously demonstrated that *C. fimbriata* administration prevented hyperglycemia, partially prevented hyperinsulinemia and significantly reduced plasma glucose levels (Sudhakara et al., 2014).

In the present study, the HF + CFE tended to have an improved glucose metabolism compared to the HF group, although not statistically significant ($p = 0.08$). Two other animal studies have observed a significant reduction in plasma glucose in *C. fimbriata* treated diabetic induced rodents (Somnath et al., 2012, Jagtap et al., 2013). The mechanism of hypoglycemic activity of *C. fimbriata* is yet to be determined. However, as *C. fimbriata* contains many chemical constituents including pregnane glycosides, flavonoids, megastigmane glycosides, bitter principles and saponins, it is not surprising that any of these secondary metabolites singly or in combination could be responsible for the hypoglycemic activity. A possible mechanism by which this botanical extract may decrease blood glucose is through either stimulating pancreatic secretion of insulin from β-cells of islets of Langerhans or via enhancing utilization of glucose in muscle and inhibiting glucose output in the liver. The proposed anti-diabetic mechanism of *C. fimbriata* is similar to that of *Caralluma edulis* extract (Sayantan and Abhishek, 2012), *Calocybe indicia* (Paramasivam and Shanmugasundaram, 2013), *Helianthus annuus* (Shivani S and Sunil, 2013), *Swertia chirayita Andrographis paniculata* (Vinod et al., 2013), and *Xanthosoma sagittifolium* (Shajeela et al., 2013).

There is also a strong relationship between insulin resistance, visceral adiposity and hypertension (Bloomgarden, 2002). According to the Framingham study, risk estimates show that approximately 80 % of hypertension in men and 65 % in women can be attributed to obesity (Garrison et al., 1987). Furthermore, low adiponectin levels seen in obese individuals are associated with the development of insulin resistance and can lead to vascular changes thereby providing a background into hypertension (Fesus et al., 2007). The present study is the first animal model to measure systolic and diastolic blood pressure to investigate the effect of *C. fimbriata* extract on hypertension in rats with diet induced obesity.

Our study failed to demonstrate that *C. fimbriata* is capable of improving blood pressure in rats with diet induced obesity. We hypothesised that *C. fimbriata* would have a positive effect on blood pressure due to the flavonoids present in the botanical extract (Salvamani et al., 2014). It has been shown in previous studies that plant extracts rich in flavonoids may be potential natural angiotensin converting enzyme (ACE) inhibitors (Balasuriya and Rupasinghe, 2012). Currently, there are an increasing number of experimental and epidemiological studies that have demonstrated a correlation between supplements rich in flavonoids and the protection against CVD and atherosclerosis. Furthermore, flavonoids inhibit lipid peroxidation, platelet aggregation and the activity of enzyme systems, which include cyclooxygenase and lipoxygenase. The biological mechanisms involved in the modulation of vascular function and blood pressure by the flavonoids is linked with the action of nitric oxide. The renin-angiotensin system is regulated in endothelial cells which may involve the control of nitric oxide production. ACE (enzyme that plays a critical role in the regulation of renin-angiotensin system) is a zinc-containing peptidyl dipeptide hydrolase (Strittmatter and Snyder, 1986). There are three known parts to the active site of ACE: a pocket that accommodates a hydrophobic side chain of C-terminal amino acid residues; a carboxylate binding functionality for instance the guanidinium group of arginine; and finally a zinc ion that coordinates to the carbonyl of the penultimate peptide bond of the substrate, which results in the carbonyl group becoming polarized and is therefore exposed to a nucleophilic attack (Loizzo et al., 2007). Thus, it may be suggested that some flavonoids elicit in vitro activity through the formation of chelate complexes found in the active centre of ACE. Due to the established inhibitory activities of flavonoids observed in other botanical extracts is it of importance to investigate the potential inhibitory activity to clarify if *C. fimbriata* extract has potential anti-hypertensive properties. The Caralluma species is well known for its antioxidant properties, with potent radical and lipid peroxide scavenging

activity (Tatiya et al., 2010). This potential anti-oxidative property of *C. fimbriata* may provide a new target for the management of hypertension. However, further research needs to be undertaken on the role of *C. fimbriata* rich in flavonoids through investigating the potential effects on vascular structure, endothelial function and oxidative status in hypertensive and normotensive rodents. An alternative method of measuring blood pressure could also be adopted such as implantable radio telemetry. This method measures direct chronic continuous blood pressure via implantable radio telemetry devices (Huetteman and Bogie, 2009).

5.7 Conclusion:

Overall the evidence presented in this study and previous work on *C. fimbriata* extract as an appetite suppressant and weight management aid is inconsistent. The results of this study have shown that *C. fimbriata* extract does not effectively reduce the major components of metabolic syndrome including excess central adiposity, hypertension, insulin resistance and hyperlipidemia. Further studies are needed to better clarify the underlying mechanisms that are involved in eliciting potential beneficial effects of *C. fimbriata* extract in the pathogenesis of metabolic and cardiovascular disorders.

CHAPTER 6: EFFECTS OF CARALLUMA FIMBRIATA AND CITRUS SINENSIS SUPPLEMENTATION ON OBESITY, METABOLIC SYNDROME AND ATHEROSCLEROSIS INDICES IN OVERWEIGHT AND OBESE SUBJECTS: A RANDOMISED, DOUBLE BLIND, PLACEBO CONTROLLED TRIAL

6.1 Abstract

Objective: Visceral adiposity is a major component of the metabolic syndrome and is associated with other cardio-metabolic diseases including type 2 diabetes and CVD. Currently there is a lack of evidence to support the use of natural botanical supplements for the management and treatment of metabolic and cardiovascular disorders. Therefore the aim of this study was to investigate the efficacy of two natural botanical supplements namely, *Caralluma fimbriata* extract and *Citrus sinensis* extract (Moro variety) for the alleviation of risk factors associated with metabolic and cardiovascular risk factors in overweight and obese Australian adults.

Design: This was a randomised, double blind placebo controlled clinical trial. Participants were randomly assigned into four groups which included: Group 1 – *C. fimbriata* (1g/day) plus *C. sinensis* (500 mg/day), Group 2 – *C. fimbriata* (1g/day), Group 3 – *C. sinensis* (500 mg/day) and Group 4 – Placebo (100 % maltodextrin). The eligibility criteria for the study included: a BMI greater than 25 kg/m^2 or a waist circumference > 94 cm (male), > 80 cm (female). Ninety participants aged between 20-60 years were recruited and randomized for the trial, however only 59 completed the 12-week study conducted at Victoria University. In addition to supplementation, participants were provided with fortnightly nutrition advice, but were asked to maintain their usual physical activities during the intervention period.

Results: The results of 59 participants (Mean age: 46.6 years; 19 males, 40 females; BMI: 34.3 kg/m2) were analysed (Placebo, n = 13; *C. fimbriata*, n = 16; *C. sinensis*, n = 16; *C.*

fimbriata plus *C. sinensis*, n = 14). A significant time effect was observed in waist circumference ($p = 0.001$) (mean reduction of 2.6 cm), hip circumference ($p = 0.001$) (mean reduction of 2.4 cm), BMI ($p = 0.001$) (mean reduction of 0.70 kg/m^2), and percentage body fat ($p = 0.02$) (mean reduction of 1.82%) from baseline to week 12 in each group. Also, there was a significant time effect ($p < 0.05$) in the FFQ data for energy, total fat, saturated fat, polyunsaturated fat, monounsaturated fat, protein, carbohydrate, fibre, sugar, and salt ($p < 0.05$). Furthermore, there was a significant time effect for all blood lipid profile parameters including Total cholesterol ($p = 0.001$), LDL cholesterol ($p = 0.001$), HDL cholesterol ($p = 0.001$), triglycerides ($p = 0.001$), HDL: LDL ratio ($p = 0.001$), atherogenic index ($p = 0.001$), cholesterol: HDL ratio ($p = 0.001$) and plasma CRP levels ($p = 0.01$). However, there was no treatment effect observed for all data on body composition, dietary intake, cardiovascular parameters, appetite sensations and lipid profile ($p > 0.05$).

Conclusion: This study has demonstrated that supplementation with *C. fimbriata* extract and/or *C. sinensis* extract in addition to a healthy diet were not effective in improving health outcomes. Following the hypocaloric diet significantly improved body composition parameters, reduced energy intake and significantly altered the lipid profile. Therefore the therapeutic use of these botanical extracts to effectively manage metabolic and cardiovascular disorders is not convincing.

Name of trial registry: Australian and New Zealand Clinical Trials Registry

Registration number: ACTRN12613000272796

6.2 Introduction

According to the World Health Organization, at least 2.8 million people die each year due to being overweight or obese, in addition to an estimated 35.8 million of global disability-adjusted life years (DALYs) are a result of being overweight or obese (World Health Organisation., 2015). Overweight or obesity has led to an increase in the development of metabolic syndrome risk factors including insulin resistance, high cholesterol and triglycerides and high blood pressure. Excess body fat particularly central adiposity is a major risk factor for cardiovascular disease, type 2 diabetes, musculoskeletal conditions and some cancers (Haslam and James, 2005). According to the Australian Bureau of Statistics, the age-adjusted prevalence of overweight and obesity in Australian adults was 63.4 % (35 % overweight; 28.3 % obese) in 2011-2012 respectively (World Health Organisation., 2015). Thus coping with obesity is an important public health issue in Australian adults. Various factors that contribute to the rise in obesity prevalence include: genetic, behavioural, environmental, physiological, social and cultural factors which leads to energy imbalance, whilst promoting excess body fat accumulation (National Health and Medical Research Council., 2013). Therefore, appropriate interventions in the form of weight reduction, modifications in dietary habits, promoting physical activity and overall wellbeing are needed in the prevention and onset of overweight and obesity, as well as their related noncommunicable diseases in order to reduce the burden of disease at the global, regional and local level.

The use of plant extracts may have a potential therapeutic role in the prevention and management of obesity and associated metabolic abnormalities (Hasani-Ranjbar et al., 2013). Traditional health care systems such as herbal medicine are now widespread in developed

countries. Furthermore, the approach in trying to combat obesity has been influenced by a growing interest in complementary and alternative medicine. Due to the increase in the market status of these plant extracts for the management and treatment of obesity and associated metabolic conditions, research has intensified in this field with the focus on the chemical constituents of these bioactive crude extracts.

C. fimbriata extract is a succulent shrub that belongs to the Apocynaceae family under the subfamily Asclepiadoideae (Milkweed family). The plant is a tender succulent that is endemic to India, Pakistan, the Canary Islands, Arabia, southern Europe, Sri Lanka and Afghanistan and flourishes in the wild. *C. fimbriata* is the most prevalent of the *Caralluma* genus and it is planted as a roadside shrub and boundary marker in gardens in Andhra Pradesh, Karnataka, and Tamil Nadu of India and grows wild in urban centres (Kuriyan et al., 2007). Traditionally, the edible wild succulent has been used for many centuries in native Indian diets with claims in folklore of hunger suppressing activity. The key phytochemical ingredients in *C. fimbriata* include pregnane glycosides, saponin glycosides, flavonoids and bitter principles. The appetite suppressing properties of *C. fimbriata* could be attributed to the pregnane glycosides, which are a rich source in the *Caralluma* species (Komarnytsky et al., 2013). There have only been two randomised controlled trials (RCTs) conducted on the efficacy of *C. fimbriata* extract on its appetite suppressing and metabolic effects. Kuriyan et al (2007) demonstrated that oral administration of *C. fimbriata* extract for two months significantly reduced appetite and waist circumference. In chapter five of this thesis (pilot study) we reported a significant decline in waist circumference after 12-weeks of *C. fimbriata* treatment which is in support of the findings by Kuriyan et al (2007). These preliminary data suggest that further studies are needed to better clarify the potential therapeutic benefits of *C.*

fimbriata extract on obesity and the metabolic abnormalities associated with this prevalent condition.

Another botanical extract used for the treatment of obesity is known as *Citrus sinensis* extract. The Moro variety is thought to have originated from Sicily and is said to be the most colourful of the red orange varieties. The orange has a deep red flesh, with the intense red to purple pigment making the fruit more attractive. The red colouration of the fruit is due to the presence of anthocyanins (flavonoid). Anthocyanins are polyphenolic compounds that have been associated with the amelioration of insulin resistance and obesity in rodents (Tsuda et al., 2003, Jayaprakasam et al., 2006, Titta et al., 2010). Anthocyanins have been reported to act as antioxidants (Lo scalzo, 2004) through the reduction in oxidative stress (Shih et al., 2007), in addition to blocking inflammation through normalizing adipocytokine expression and have been shown to exert anti-tumour activities (Guo et al., 2008). In humans, administration of *C. sinensis* extract has been found to reduce inflammatory markers (Buscemi et al., 2012), biomarkers of oxidative stress (Bonina et al., 2005) and improve body mass index (Dallas et al., 2008). However, the efficacy of *C. sinensis* extract on metabolic syndrome has yet to be explored. Therefore, in view of the beneficial metabolic effects of *C. fimbriata* extract observed in the previous studies and the promising anti-obesity and anti-inflammatory effects of *C. sinensis* extract, it is of interest to evaluate the joint administration of these two supplements on obesity, metabolic syndrome and atherosclerotic indices in overweight and obese Australian adults.

6.3 Materials and Methods

6.3.1 Experimental design, participant recruitment and randomisation

This study was a randomized, double-blinded, placebo controlled trial, involving 59 human volunteers, between the ages of 20 and 60 years, with a mean age of 47. The complete study group involved 19 males and 40 females. Recruitment of participants and eligibility criteria are described in section 3.1. The final number of participants in each group are as follows: Placebo (Group 4) (n = 13; 9 females, 4 males), *C. fimbriata* (Group 2) (n = 16; 12 females, 4 males), *C. sinensis* (Group 3) (n = 16; 10 females, 6 males), and *C. fimbriata* + *C. sinensis* (Group 1) (n = 14; 9 females, 5 males). For randomisation, participants were coded and allocated into four groups based on their physical characteristics including age, body weight, height, BMI, waist and hip circumference and WHR. Further details of the randomisation process are presented in section 3.1.

The study was approved by the Human Research Ethics Committee of Victoria University, Australia (HRETH 12/264) and registered by ANZCTR. At the beginning of the study all eligible volunteers were informed about the details of the study including that they would be randomly assigned into one of the four groups. Formal consent was obtained from all participants.

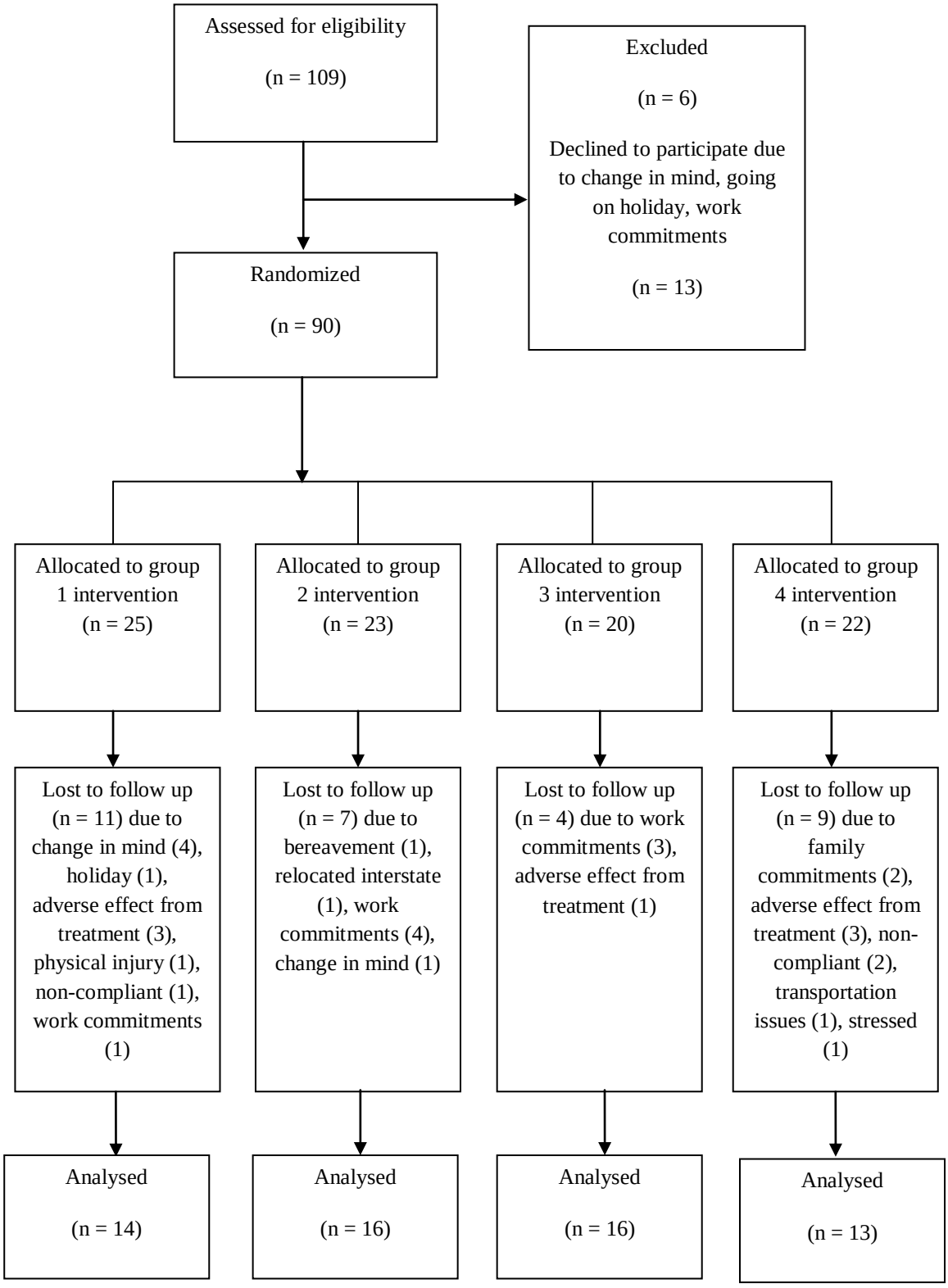


Figure 6.1: The study flow diagram of the progress of the four phases of the study which include: enrolment, intervention allocation, follow-up and data analysis.

6.3.2 Administration of botanical extracts

The test articles were administered as two equally divided doses (in capsule form) 30 minutes before two meals each day for 12 weeks. The administration of the test supplements is presented in Table 6.1. Further details on the administration, dosage and monitoring of ingestion of capsules is outlined in section 3.1.1

Table 6.1: Group assignment, administration and dosage of test articles

Group	Administration	Dose
Group 1 *C. fimbriata* + *C. sinensis*	Two capsules orally administered 30 minutes before meals twice daily	1 g *C. fimbriata* + 500 mg *C. sinensis*
Group 2 *C. fimbriata*		1 g *C. fimbriata*
Group 3 *C. sinensis*		500 mg *C. sinensis*
Group 4 Placebo		100 % maltodextrin

6.3.3 Outcome measures

Participants were asked to attend the Victoria University Nutrition Clinic, Melbourne, Australia for fortnightly nutrition consultations. During the intervention period dietary intake and exercise were controlled. Participants were asked to maintain their usual participation in physical activities during the trial period. Physical activity was monitored by providing volunteers with a physical activity calendar and a physical activity questionnaire (long, self-administered IPAQ) was provided at baseline and post intervention (as outlined in section 3.2.4). Dietary intake was monitored through 3-day food diaries submitted monthly and a food frequency questionnaire (DQESV2) was completed at baseline and post intervention (as outlined in section 3.2.4). The participants also followed a hypocaloric diet during the

intervention period (deficit of approximately 500kcal/day of estimated energy requirements). Anthropometric measurements including body weight, height, waist and hip circumference, waist to hip ratio (refer to section 3.2.1), as well as blood pressure and heart rate were taken during each nutrition consultation (section 3.2.2). Appetite sensations and hunger were assessed via the visual analogue scales method at baseline and post intervention (as outlines in section 3.2.3). Body composition (percentage body fat) was measured using the 3D laser scanner at baseline and post intervention (as outlined in section 3.2.1). At baseline and at the end of the trial period, biochemical parameters including blood lipid profile, CRP, leptin, atherogenic index of plasma, insulin and ghrelin levels were analysed, as outlined in section 3.3 of this thesis.

6.4 Statistical Analysis:

All data were presented as the mean and standard error and were analysed using SPSS package, version 22 (SPSS, Chicago, IL, USA). Target sample size was calculated to include a minimum of 13 participants per group to detect significant differences in appetite, one of the main measured outcomes with 90 % power (using the mean change of 25 and standard deviation of 20), based on the previous findings of Kuriyan et al (2007). One way ANOVA was performed to compare all baseline data between groups. Mixed model ANOVA was used to analyse the effects of the intervention, time, and the interaction between the intervention and time with pairwise comparisons (adjusted for multiple comparisons by Bonferroni's post-hoc test). When the interaction and/or the main effects were significant, means were compared using Tukey's multiple comparison post hoc test. The significant level was set as $p < 0.05$. The precision of the primary and secondary outcomes for each group were calculated using 95 % confidence intervals.

6.5 Results:

6.5.1 Study participants

There were 109 volunteers screened for enrolment into the study, with only six volunteers excluded and 13 volunteers who declined to participate. A total of 90 volunteers were randomized into four groups to receive supplemental treatments or placebo. The data analysis was completed for 14 participants in group 1, 16 participants in groups 2 & 3, and a total of 13 participants in group 4 (Figure 6.1). In addition, there was no breach of the blinding process identified throughout the intervention period.

6.5.2 Participant characteristics

Baseline general characteristics of participants that completed the study are shown in Table 6.2. There were no significant differences recorded in the baseline characteristics such as age (p = 1.0), body weight (p = 1.0), body mass index (p = 0.99), waist circumference (p = 0.95), hip circumference (p = 0.97) and waist to hip ratio (p = 0.82) between all groups.

Table 6.2: Baseline physical characteristics of participants

Variable	*C. fimbriata* + *C. sinensis* (n = 14)	*C. fimbriata* (n = 16)	*C. sinensis* (n = 16)	Placebo (n = 13)	P value
Age (yrs)	46.2 ± 3.40	47.1 ± 2.60	46.6 ± 3.10	46.5 ± 2.17	1.0
Body weight (kg)	97.53 ± 6.17	98.56 ± 5.98	97.22 ± 6.29	99.2 ± 7.94	1.0
Body mass index (kg/m^2)	34.76 ± 2.14	34.43 ± 1.50	33.95 ± 1.77	34.08 ± 2.22	0.99
Waist circumference (cm)	108.91 ± 4.35	111.30 ± 4.67	109.06 ± 3.65	107.67 ± 5.38	0.95
Hip circumference (cm)	116.66 ± 4.27	116.86 ± 2.99	118.5 ± 3.55	116.17 ± 4.44	0.97
Waist to hip ratio	0.94 ± 0.03	0.99 ± 0.02	0.95 ± 0.01	0.93 ± 0.03	0.82

Values are expressed as mean ± SEM, n = number of participants, yrs = years, kg = kilograms, kg/m^2 = kilograms per meters squared, cm = centimetres. There were no significant differences between groups for all baseline variables (p >0.05).

6.5.3 Anthropometric parameters

Baseline and post-intervention anthropometric parameters including body weight, BMI, waist circumference, hip circumference, WHR & percentage body fat are presented in Table 6.3. A significant time effect was observed in waist circumference ($p = 0.001$) (mean reduction of 2.6 cm), hip circumference ($p = 0.001$) (mean reduction of 2.4 cm), BMI ($p = 0.001$) (mean reduction of 0.70 kg/m^2), and percentage body fat ($p = 0.02$) (mean reduction of 1.82%) from baseline to week 12 in each group. There were no significant differences between groups for all anthropometric variables.

6.5.4 Cardiovascular parameters

The cardiovascular parameters including heart rate, systolic and diastolic blood pressure are presented in Table 6.4. There were no significant differences between groups in systolic BP and diastolic BP ($p > 0.05$). There was a significant time effect ($p < 0.05$) in heart rate from baseline to week 12, with a mean increase of 5.87 beats/minute.

6.5.5 Dietary assessment

Food intake was assessed by multiple methods including 3-day food diaries, FFQs and via the VAS method. Significant findings were observed in the food frequency questionnaire data which is presented in Table 6.6. There was a significant time effect ($p < 0.05$) in the FFQ data for energy, total fat, saturated fat, polyunsaturated fat, monounsaturated fat, protein, carbohydrate, fibre, sugar, and salt. There were significant interactions between group and time for carbohydrate intake and polyunsaturated fat intake ($p = 0.05$ and $p = 0.02$

respectively), however there were no significant between group differences. In addition, there were no significant differences observed for all food intake data assessed via 3-day food diary records ($p > 0.05$).

Food & energy consumption of the test breakfast and satiety quotient (SQ) for the degree of hunger at baseline and post intervention is presented in Table 6.7. No significant differences were observed for all food and energy consumption of the test breakfast and satiety quotient ($p > 0.05$). Ratings of appetite and mood states after the test breakfast at baseline & post-intervention are presented in Figure 6.2 a - n. Overall, no significant differences were observed for all ratings of appetite and mood states following the test breakfast ($p > 0.05$).

6.5.6 Lipid & carbohydrate metabolism parameters

The biochemical parameters (blood lipid profile, atherogenic index and plasma levels of CRP, insulin, blood glucose, HOMA, leptin and ghrelin) are presented in Table 6.8 and 6.9. There was a significant time effect for all blood lipid profile parameters including total cholesterol ($p = 0.001$), LDL cholesterol ($p = 0.001$), HDL cholesterol ($p = 0.001$), triglycerides ($p = 0.001$), HDL:LDL ratio ($p = 0.001$), atherogenic index ($p = 0.001$), cholesterol:HDL ratio ($p = 0.001$) and plasma CRP levels ($p = 0.01$). There were also significant interactions for total cholesterol ($p = 0.001$), LDL cholesterol ($p = 0.001$), leptin ($p = 0.04$), LDL: HDL ratio ($p = 0.001$), CRP ($p = 0.001$) and cholesterol: HDL ratio ($p = 0.001$). Total cholesterol, LDL cholesterol, LDL/HDL ratio & cholesterol/HDL ratio was significantly lower in the placebo group compared to all other groups ($p < 0.05$). HDL cholesterol was significantly lower in the *C. fimbriata* group compared to all other groups ($p < 0.05$). There were no significant treatment effects observed for lipid and carbohydrate metabolism parameters.

6.5.7 Physical activity assessment

The self-reported physical activity on the International physical activity questionnaire by domain is presented in Table 6.10. There was a significant interaction for total domestic and garden MET-mins/week ($p = 0.01$). The self-reported physical activity on the International physical activity questionnaire: Total scores for all walking, moderate & vigorous physical activities, total physical activity score, total sitting score & classification of physical activity score is presented in Table 6.11. There was a significant interaction for total walking MET/mins/week ($p = 0.03$). It was also found that there was a significant time effect observed for total sitting and average sitting mins/week ($p = 0.02$). There were no significant differences between groups for all physical activity measurements.

Table 6.3: Anthropometric data before and after treatment

Variable	Baseline	Week 12	Baseline	Week 12	Baseline	Week 12	Baseline	Week 12	P value	
	C. fimbriata + C. sinensis		*C. fimbriata*		*C. sinensis*		Placebo			
BW (kg)	97.53 ± 6.17	96.2 ± 6.24	98.56 ± 5.98	95.88 ± 5.84	97.22 ± 6.29	96.02 ± 6.37	99.2 ± 7.94	96.61 ± 0.83	Group Time Interaction	1.0 0.00 0.27
BMI (kg/m^2)	34.76 ± 2.14	34.20 ± 0.31	34.43 ± 1.50	33.50 ± 1.47	33.95 ± 1.77	33.53 ± 1.81	34.08 ± 2.22	33.19 ± 0.28	Group Time Interaction	0.99 0.00 0.33
WC (cm)	108.91 ± 4.35	107.57 ± 4.35	111.30 ± 4.67	107.84 ± 4.26	109.06 ± 3.65	106.03 ± 4.22	107.67 ± 5.38	105.27 ± 5.11	Group Time Interaction	0.97 0.00 0.55
HC (cm)	116.66 ± 4.27	114.71 ± 4.38	116.86 ± 2.99	114.69 ± 2.98	118.5 ± 3.55	114.47 ± 3.21	116.17 ± 4.44	114.92 ± 4.59	Group Time Interaction	1.0 0.00 0.24
WHR	0.94 ± 0.03	0.96 ± 0.04	0.99 ± 0.02	0.99 ± 0.02	0.95 ± 0.01	0.97 ± 0.03	0.93 ± 0.03	0.94 ± 0.04	Group Time Interaction	0.89 0.77 0.70
BF (%)	45.20 ± 4.21	43.37 ± 4.18	43.66 ± 3.48	40.46 ± 2.94	41.96 ± 2.60	41.68 ± 2.73	42.0 ± 3.41	40.0 ± 3.76	Group Time Interaction	0.54 0.02 0.54

Values are expressed as mean ± SEM. BW = body weight, BMI = Body mass index, WC = waist circumference, HC = hip circumference, WHR = waist to hip ratio, BF = 3-dimentional body fat.

Table 6.4: Physiological parameters at baseline and post-intervention

Variable	Baseline	Week 12	Baseline	Week 12	Baseline	Week 12	Baseline	Week 12	P value	
	C. fimbriata + C. sinensis		*C. fimbriata*		*C. sinensis*		Placebo			
Systolic BP (mmHg)	132.36 ± 4.24	132.39 ± 3.78	133.94 ±3.65	127.38 ±3.75	130.91 ± 5.30	133.0 ± 5.63	129.50 ± 5.18	130.23 ± 4.19	Group Time Interaction	0.97 0.61 0.32
Diastolic BP (mmHg)	84.11 ± 2.80	79.82 ± 2.43	84.08 ± 2.75	80.56 ± 12.21	81.03 ± 2.79	81.0 ± 2.98	79.46 ± 3.38	80.62 ± 2.07	Group Time Interaction	0.93 0.20 0.39
Heart rate (beats/min)	68.21 ± 3.22	76.21 ± 4.28	69.25 ± 3.38	75.31 ± 2.65	69.07 ±2.61	76.81 ± 3.34	71.50 ± 3.68	73.15 ± 2.94	Group Time Interaction	1.0 0.00 0.49

Values are expressed as mean ± SEM. BP = Blood pressure, mmHg = Millilitre of mercury, min = minute.

Table 6.5: Food intake assessment (3-day food diary) at baseline and post-intervention

Variable	Baseline	Week 12	Baseline	Week 12	Baseline	Week 12	Baseline	Week 12	P value	
	C. fimbriata + C. sinensis		*C. fimbriata*		*C. sinensis*		Placebo			
Energy (kJ/day)	7985.99 ± 518.89	7215.53 ± 624.12	7814.50 ± 473.91	7013.63 ± 425.87	7065.54 ± 430.76	6820.04 ± 562.61	8950.63 ± 1443.48	7716.73 ± 773.44	Group Time Interaction	0.33 0.10 0.89
Protein (g/day)	94.34 ± 7.36	87.21 ± 9.98	101.12 ± 7.77	97.44 ± 4.76	82.69 ± 6.18	95.89 ± 4.75	120.07 ± 23.00	91.72 ± 10.14	Group Time Interaction	0.35 0.32 0.18
Fat (g/day)	82.37 ± 7.60	68.50 ± 7.86	66.16 ± 5.15	58.78 ± 6.35	57.19 ± 4.74	63.41 ± 4.29	89.19 ± 22.66	67.31 ± 9.37	Group Time Interaction	0.21 0.17 0.47
Sat fat (g/day)	27.90 ± 2.45	25.48 ± 3.40	26.94 ± 3.25	20.97 ± 2.21	20.39 ± 1.57	22.03 ± 2.06	37.72 ± 14.56	25.48 ± 3.92	Group Time Interaction	0.41 0.25 0.45
CHO (g/day)	170.13 ± 10.81	170.0 ± 12.87	202.28 ± 14.12	175.92 ± 12.28	193.88 ± 12.11	177.58 ± 12.69	194.73 ± 16.62	199.48 ± 17.76	Group Time Interaction	0.37 0.19 0.41
Sugar (g/day)	67.11 ± 5.24	71.26 ± 4.92	93.90 ± 8.66	81.08 ± 8.01	83.31 ± 7.94	69.02 ± 6.24	82.30 ± 9.11	76.39 ± 7.02	Group Time Interaction	0.13 0.07 0.33
Alcohol (g/day)	10.91 ± 4.44	5.76 ± 3.08	0.49 ± 0.48	2.83 ± 2.08	4.26 ± 2.18	1.49 ± 0.90	4.31 ± 2.24	4.18 ± 1.71	Group Time Interaction	0.13 0.26 0.19
Fibre (g/day)	21.57 ± 1.79	21.77	21.73 ± 2.45	19.62 ± 1.02	21.43 ± 1.06	25.67 ± 1.94	19.38 ± 2.05	24.88 ± 2.27	Group Time Interaction	0.61 0.14 0.11
Salt (mg/day)	2631.21 ± 365.89	2316.76 ± 231.47	2044.02 ± 136.20	1954.06 ± 166.72	2167.49 ± 176.81	2437.94 ± 224.08	2968.22 ± 570.42	2401.52 ± 328.02	Group Time Interaction	0.29 0.36 0.40

Values are expressed as mean ± SEM. kJ = kilojoules, g = grams, mg = milligrams, sat fat = saturated fat, CHO = carbohydrate.

Table 6.6: Nutrient intakes from Food Frequency Questionnaire at baseline & post-intervention

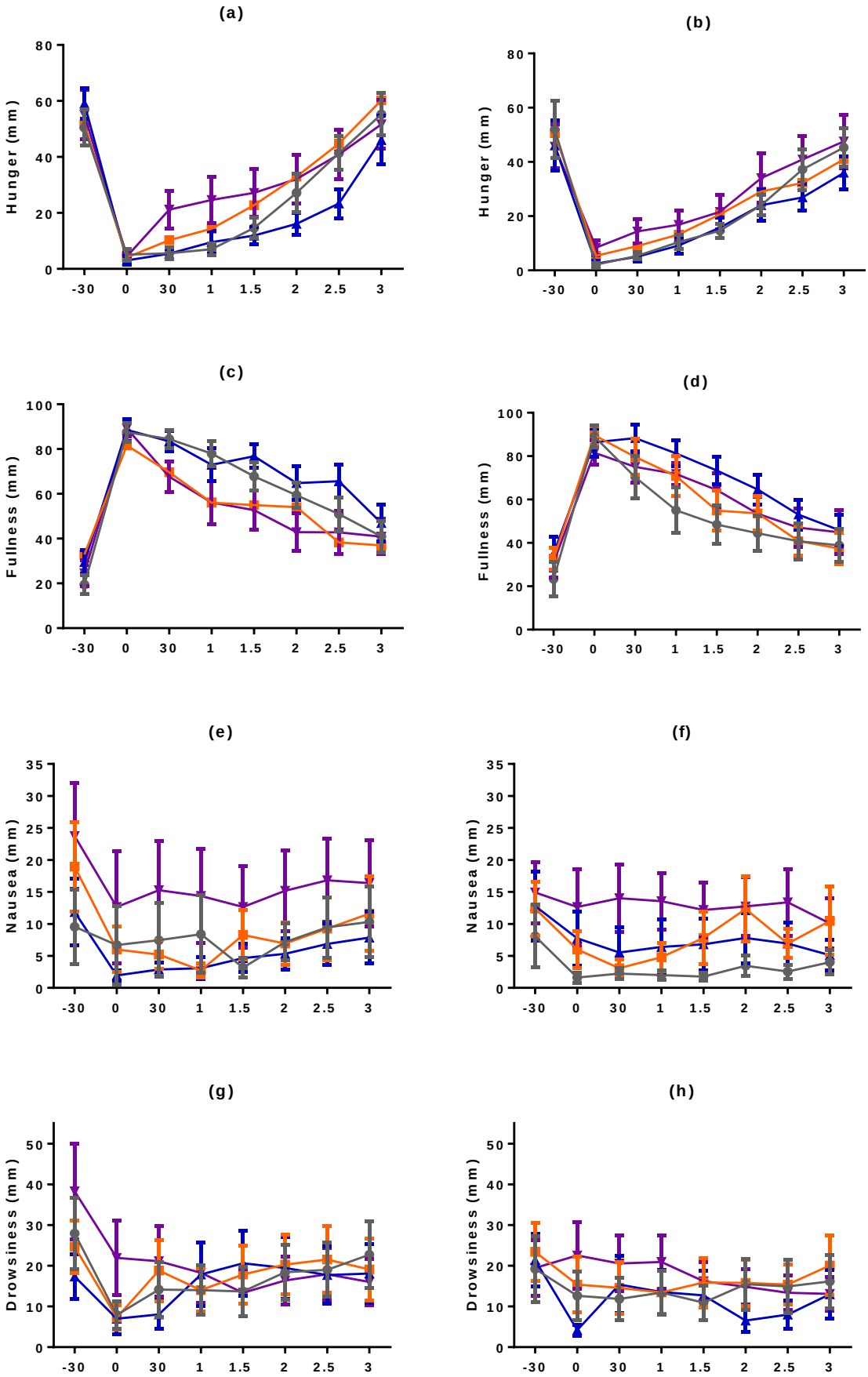
Variable	Baseline	Week 12	Baseline	Week 12	Baseline	Week 12	Baseline	Week 12	P value	
	C. fimbriata + C. sinensis		*C. fimbriata*		*C. sinensis*		Placebo			
Energy (kJ/day)	10837.43 ± 2869.15	6494.61 ± 694.71	10260.20 ± 1651.90	6656.23 ± 715.57	8421.64 ± 843.30	5403.30 ± 427.85	6751.24 ± 826.13	7524.36 ± 1233.53	Group Time Interaction	0.61 0.00 0.07
Protein (g/day)	133.35 ± 34.71	85.16 ± 7.85	118.41 ± 23.16	86.05 ± 10.29	95.83 ± 7.95	71.34 ± 6.04	80.12 ± 9.66	87.35 ± 15.93	Group Time Interaction	0.48 0.01 0.21
Fat (g/day)	116.74 ± 34.82	63.15 ± 7.81	109.36 ± 18.45	63.89 ± 7.71	87.49 ± 9.96	50.20 ± 4.69	68.81 ± 9.25	74.57 ± 11.96	Group Time Interaction	0.59 0.00 0.09
Sat fat (g/day)	47.53 ± 15.04	24.05 ± 3.02	42.83 ± 7.57	23.68 ± 3.10	33.33 ± 3.36	18.16 ± 1.66	28.36 ± 4.70	28.74 ± 5.26	Group Time Interaction	0.55 0.00 0.15
Poly fat (g/day)	16.22 ± 4.12	9.61 ± 1.48	17.14 ± 2.90	10.94 ± 1.20	14.51 ± 2.23	8.57 ± 1.14	9.75 ± 1.23	11.71 ± 2.0	Group Time Interaction	0.66 0.00 0.02
Mono fat (g/day)	43.17 ± 13.23	23.66 ± 2.83	39.90 ± 7.04	23.47 ± 2.96	32.19 ± 4.08	18.79 ± 1.92	24.65 ± 3.33	27.23 ± 4.27	Group Time Interaction	0.59 0.00 0.10
CHO (g/day)	254.56 ± 61.34	162.29 ± 19.66	251.74 ± 37.54	169.32 ± 17.86	212.74 ± 22.55	139.08 ± 10.83	170.29 ± 22.23	195.55 ± 32.81	Group Time Interaction	0.73 0.00 0.05
Sugar (g/day)	104.20 ± 22.76	78.14 ± 8.62	105.87 ± 12.79	81.25 ± 7.43	85.42 ± 8.35	64.32 ± 4.91	77.82 ± 10.77	78.08 ± 7.35	Group Time Interaction	0.41 0.01 0.43
Fibre (g/day)	25.91 ± 5.99	20.77 ± 2.86	24.19 ± 3.49	19.27 ± 1.82	23.03 ± 2.34	18.56 ± 1.59	20.05 ± 2.20	22.71 ± 3.19	Group Time Interaction	0.93 0.02 0.12
Salt (mg/day)	3484.17 ± 865.60	2076.65 ± 226.33	3281.99 ± 626.77	2154.40 ± 301.06	2867.65 ± 304.30	1835.39 ± 175.95	2158.39 ± 311.05	2508.05 ± 528.53	Group Time Interaction	0.79 0.00 0.06

Values are expressed as mean ± SEM. kJ = kilojoules, g = grams, mg = milligrams, sat fat = saturated fat, mono fat = monounsaturated fat, poly fat = polyunsaturated fat.

Table 6.7: Food & energy consumption of test breakfast and satiety quotient (SQ) for the degree of hunger at baseline and post intervention

Variable	Baseline	Week 12	Baseline	Week 12	Baseline	Week 12	Baseline	Week 12	P value	
	C. fimbriata + C. sinensis		*C. fimbriata*		*C. sinensis*		Placebo			
Total food consumption (g)	657.21 ± 45.98	698.89 ± 84.84	676.90 ± 78.16	649.63 ± 91.54	934.91 ± 89.70	729.34 ± 85.45	735.72 ± 83.94	677.89 ± 103.32	Group Time Interaction	0.32 0.16 0.23
Total energy consumption (kJ)	2390.39 ± 212.33	2143.32 ± 265.23	2512.81 ± 172.60	2102.79 ± 192.36	2656.07 ± 294.84	2275.41 ± 261.96	2637.63 ± 372.72	2546.56 ± 449.64	Group Time Interaction	0.74 0.09 0.78
Time taken to eat (mins)	12.86 ± 0.99	15.07 ± 1.37	15.81 ± 1.69	14.13 ± 1.72	16.50 ± 1.38	14.44 ± 1.48	15.92 ± 5.02	13.92 ± 1.22	Group Time Interaction	0.82 0.34 0.31
SQ for degree of Hunger (mm/kcal)	4.51 ± 1.07	5.24 ± 2.23	4.23 ± 1.09	5.47 ± 1.56	7.36 ± 1.03	5.68 ± 1.55	4.85 ± 1.64	1.91 ± 2.01	Group Time Interaction	0.37 0.49 0.37

Values are expressed as mean ± SEM, kJ = kilojoules, g = grams, mins = minutes, SQ = satiety quotient, mm/kcal = milligrams per kilocalorie.


(a)
Hunger (mm)
(b)
Hunger (mm)
(c)
Fullness (mm)
(d)
Fullness (mm)
(e)
Nausea (mm)
(f)
Nausea (mm)
(g)
Drowsiness (mm)
(h)
Drowsiness (mm)
-30
0
30
1
1.5
2
2.5
3

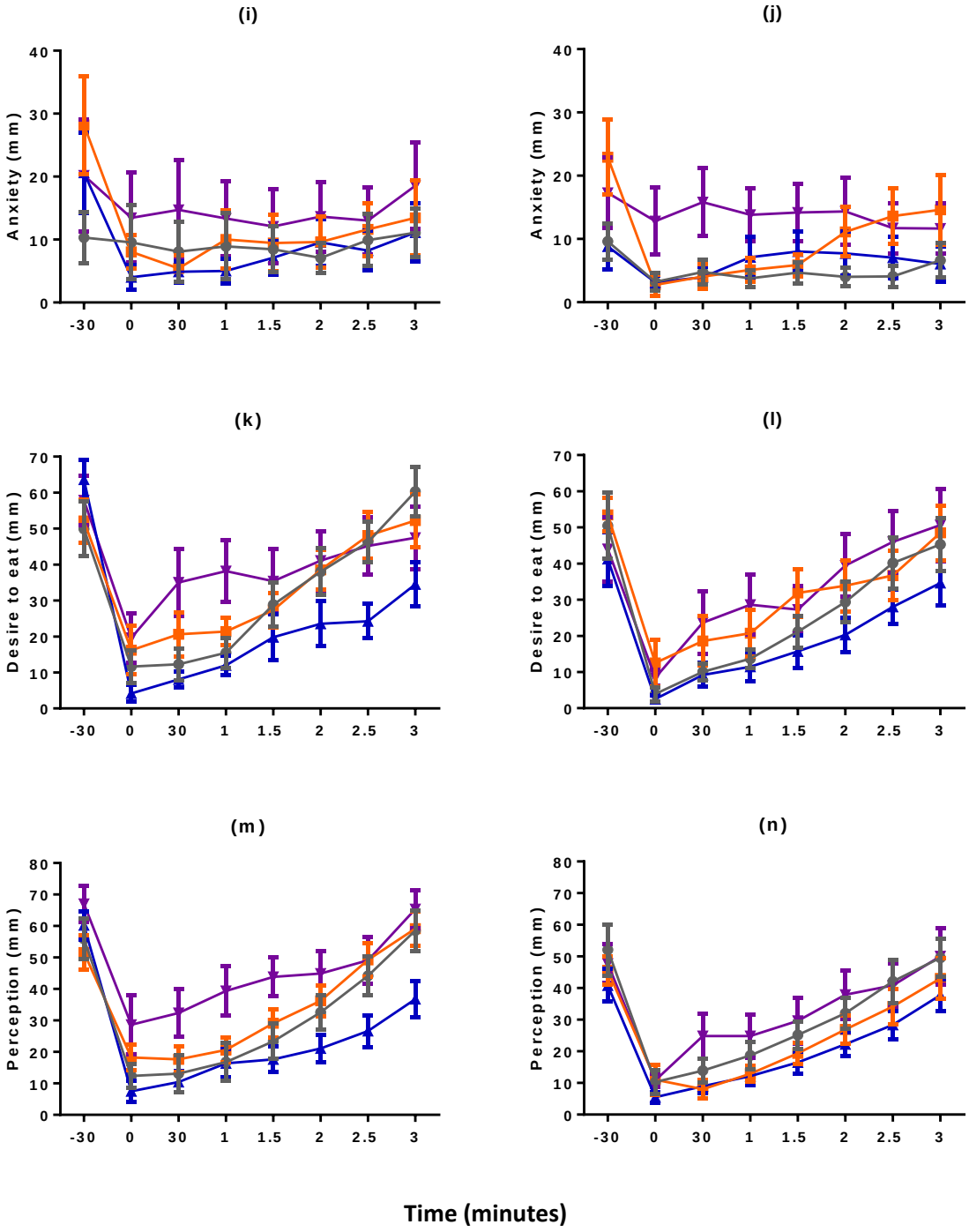


Figure 6.2 a -n: Ratings of appetite and mood states after two identical breakfast test meals at baseline & post-intervention. ● Group 1 (grey); ■ Group 2 (orange); ▲Group 3 (blue); ▼Group 4 (purple); (a, c, e, g, i, k, m) Baseline; (b, d, f, h, j, l, n) Post-intervention. Anchor points for ratings: (a, b) not hungry – hungry; (c, d) not full – full; (e, f) not nauseous – nauseous, (g, h) not drowsy – drowsy, (i, j) Calm – Anxious; (k, l) weak – strong; (m, n) none – a large amount. Values are expressed as mean ± SEM. mm = millimetres. No significant differences were observed for all ratings of appetite and mood states following the test breakfast ($p > 0.05$).

Table 6.8: Plasma glucose and blood lipid profile at baseline and post-intervention

Variable	Baseline	Week 12	Baseline	Week 12	Baseline	Week 12	Baseline	Week 12	P value	
	C. fimbriata + C. sinensis		*C. fimbriata*		*C. sinensis*		Placebo			
Fasting blood glucose (mmol/L)	5.37 ± 0.26	5.02 ± 0.29	5.62 ± 0.39	5.21 ± 0.53	5.13 ± 0.38	4.96 ± 0.35	5.04 ± 0.22	5.40 ± 0.25	Group Time Interaction	0.91 0.35 0.34
Triglycerides (mmol/L)	0.81 ± 0.12	0.91 ± 0.12	0.53 ± 0.10	0.91 ± 0.13	0.60 ± 0.13	0.81 ± 0.08	0.50 ± 0.06	0.78 ± 0.09	Group Time Interaction	0.56 0.00 0.20
Total cholesterol (mmol/L)	9.76 ± 0.63	3.98 ± 0.14^{b}	7.46 ± 0.23	3.05 ± 0.08^{b}	9.21 ± 0.62	3.45 ± 0.18^{b}	3.40 ± 0.44	2.50 ± 0.11^{a}	Group Time Interaction	0.00 0.00 0.00
HDL cholesterol (mmol/L)	2.98 ± 0.15	1.92 ± 0.10^{b}	1.71 ± 0.08	1.01 ±0.06^{a}	2.34 ± 0.16	1.42 ± 0.10^{b}	2.61 ± 0.13	1.70 ± 0.20^{b}	Group Time Interaction	0.00 0.00 0.26
LDL cholesterol (mmol/L)	6.48 ± 0.57	1.73 0.18^{b}	5.55 ± 0.19	1.70 ± 0.07^{b}	6.65 ± 0.52	1.73 ± 0.24^{b}	0.61 ± 0.4	0.51 ± 0.22^{a}	Group Time Interaction	0.00 0.00 0.00
LDL/HDL ratio (mmol/L)	2.20 ± 0.20	0.96 ± 0.13^{b}	3.32 ± 0.16	1.80 ± 0.13^{b}	2.93 ± 0.28	1.49 ± 0.38^{b}	0.23 ± 0.15	0.55 ± 0.23^{a}	Group Time Interaction	0.00 0.00 0.00
Atherogenic index (mmol/L)	-0.63 ± 0.08	-0.36 ± 0.06	-0.60 ± 0.07	-0.09 ± 0.05	-0.74 ± 0.10	-0.27 ± 0.06	-0.77 ± 0.08	-0.40 ± 0.09	Group Time Interaction	0.13 0.00 0.22
Cholesterol/HDL (mmol/L)	3.31 ± 0.19	2.14 ± 0.14^{b}	4.43 ± 0.16	3.14 ± 0.15^{b}	4.03 ±0.28	2.72 ± 0.39^{b}	1.30 ± 0.14	1.75 ± 0.26^{a}	Group Time Interaction	0.00 0.00 0.00

Values are expressed as mean ± SEM. mmol/L = millimoles per litre, HDL = High density lipoprotein, LDL = low density lipoprotein. Values in the same row with different letters indicate significant differences ($p<0.05$).

Table 6.9: Insulin, HOMA and cytokine levels at baseline and post-intervention

Variable	Baseline	Week 12	Baseline	Week 12	Baseline	Week 12	Baseline	Week 12	P value	
	C. fimbriata + C. sinensis		*C. fimbriata*		*C. sinensis*		Placebo			
Insulin (mU/L)	10.29 ± 1.52	6.82 ± 1.11	9.78 ± 1.55	10.70 ± 2.62	9.10 ± 1.90	7.42 ± 1.24	6.32 ± 1.86	6.08 ± 1.94	Group Time Interaction	0.44 0.19 0.28
HOMA	2.57 ± 0.48	1.59 ± 0.34	2.56 ± 0.44	3.24 ± 1.38	1.97 ± 0.36	1.76 ± 0.36	1.42 ± 0.45	1.46 ± 0.49	Group Time Interaction	0.38 0.77 0.49
Leptin (ng/mL)	42.86 ± 9.12	46.65	31.02 ± 6.36	32.35 ± 5.30	45.86 ± 9.14	54.09 ± 10.07	46.49 ± 8.85	34.74 ± 8.87	Group Time Interaction	0.27 0.34 0.04
Ghrelin (pg/mL)	680.32 ± 43.75	726.58	692.78 ± 54.51	644.51 ± 51.36	700.02 ± 72.17	632.60 ± 49.70	681.44 ± 52.58	696.28 ± 61.43	Group Time Interaction	0.86 0.83 0.52
CRP (ng/mL)	3.85 ±0.57	2.10 ± 0.43	2.00 ± 0.34	2.07 ±0.29	1.88 ± 0.40	1.50 ± 0.37	2.17 ± 0.54	2.25 ± 0.64	Group Time Interaction	0.41 0.00 0.00

Values are expressed as mean ± SEM. mU/L = milliunits per litre, HOMA = homeostatic model assessment, ng/mL = nanogram per millilitre, pg/mL = pictogram per millilitre, CRP = C-reactive protein.

Table 6.10: Self-reported physical activity on the International physical activity questionnaire by domain

Variable	Baseline	Week 12	Baseline	Week 12	Baseline	Week 12	Baseline	Week 12	P value	
	C. fimbriata + *C. sinensis*		*C. fimbriata*		*C. sinensis*		Placebo			
Total work MET-mins/week	1320 ± 856	3045 ± 1155	4828 ± 3033	2871 ± 1866	1883 ± 790	2739 ± 943	2298 ± 1322	2234 ± 992	Group Time Interaction	0.81 0.85 0.32
Total transport MET-mins/week	518 ± 193	553 ± 145	426 ± 147	774 ± 392	549 ± 214	380 ± 79	145 ± 105	564 ± 211	Group Time Interaction	0.76 0.25 0.38
Total domestic & garden MET-mins/week	922 ± 334	761 ± 241	1891 ± 477	1399 ± 389	936 ± 257	1106 ± 327	848 ± 165	2465 ± 654	Group Time Interaction	0.17 0.18 0.01
Total leisure-time MET-mins/week	661 ± 190	607 ± 186	683 ± 327	697 ± 204	819 ± 205	867 ± 220	743 ± 352	1156 ± 363	Group Time Interaction	0.75 0.46 0.68

Values are expressed as mean ± SEM, MET = the metabolic equivalent of task, mins = minutes.

Table 6.11: Self-reported physical activity on the International physical activity questionnaire: Total scores for all walking, moderate & vigorous physical activities, total physical activity score, total sitting score & classification of physical activity score.

Variable	Baseline	Week 12	Baseline	Week 12	Baseline	Week 12	Baseline	Week 12	P value	
	C. fimbriata + C. sinensis		*C. fimbriata*		*C. sinensis*		Placebo			
Total walking MET-mins/week	896 ± 258	1615 ± 380	2050 ± 956	1376 ± 789	1703 ± 508	1120 ± 250	1179 ± 550	1901 ± 634	Group Time Interaction	0.95 0.83 0.03
Total moderate MET-mins/week	1288 ± 368	1677 ± 411	2811 ± 674	2765 ± 932	1722 ± 433	3017 ± 977	1863 ± 729	3477 ± 857	Group Time Interaction	0.38 0.08 0.53
Total vigorous MET-mins/week	1237 ± 840	1674 ± 866	2968 ± 1643	1600 ± 973	763 ± 257	955 ± 395	991 ± 477	1040 ± 443	Group Time Interaction	0.55 0.68 0.38
Total PA MET-mins/week	3421 ± 1106	4966 ± 1335	7829 ± 3047	5741 ± 2314	4187 ± 887	5092 ± 1230	4033 ± 1319	6418 ± 1345	Group Time Interaction	0.67 0.39 0.21
Sitting total mins/week	2284 ± 241	2252 ± 295	2893 ± 373	2627 ± 447	2288 ± 283	1777 ± 234	2769 ± 307	2199 ± 292	Group Time Interaction	0.30 0.02 0.56
Average sitting total mins/day	326 ± 34	322 ± 42	413.30 ± 53.28	375.27 ± 63.89	326.79 ± 40.46	253.93 ± 33.39	395.60 ± 43.87	314.18 ± 41.70	Group Time Interaction	0.30 0.02 0.56
Categorical score (low: 1/moderate: 2 or high: 3)	2 ± 0	3 ± 0	2 ± 0	2 ± 0	2 ± 0	2 ± 0	2 ± 0	3 ± 0	Group Time Interaction	0.98 0.07 0.08

Values are expressed as mean ± SEM. MET = the metabolic equivalent of task, mins = minutes, PA = physical activity.

6.5.8 Intervention compliance and adverse events

The overall compliance of the participants to the capsule ingestion in the combination supplemental group was 90.21 %; *C. fimbriata* group, 90.84 %; *C. sinensis* group, 84.37 %; and the placebo group, 75.73 %. There were no serious adverse events reported by participants in this study. The observed minor side effects reported in the combination supplemental group (*C. fimbriata* + *C. sinensis*) include: bloating (n = 1), gastric acidity (n = 1), itchy skin (n = 2), skin rash (n = 2), abdominal pain (n = 1), constipation (n = 1), increased sweating (n = 1), dry scalp (n = 1). The minor side effects observed in the *C. fimbriata* group were: bloating (n = 2), gastric acidity (n = 1), frequent bowel movements (n = 1), dry skin (n = 1), itchy skin (n = 1), skin rash (n = 1), constipation (n = 1), diarrhoea (n = 1). The observed minor side effects reported in the *C. sinensis* group include: irregular period (n = 1) and increased blood pressure (n = 1). The side effects observed in the placebo group were: bloating (n = 1), sensation in the chest (n = 1), constipation (n = 1), nausea (n = 1) and diarrhoea (n = 1).

6.6 Discussion

In the present study, supplementation with *C. fimbriata* extract and/or *C. sinensis* extract in conjunction with a hypocaloric diet did not significantly affect weight loss or other anthropometric measures when compared to the placebo group. However a significant time effect was observed in the reduction of waist circumference, hip circumference, body weight and percentage body fat after the 12-week intervention period in all groups. Therefore it appears likely that the significant reductions in anthropometric measurements occurred due to the nutritional advice to follow a hypocaloric diet. It has been demonstrated that waist circumference is the best indicator of obesity in the clinical setting, as a larger waist circumference is more likely to be associated with an increased risk of metabolic related diseases (Bosello and Zamboni, 2000). A wealth of studies have established the relationship between abdominal fat distribution and the risk of metabolic syndrome (Li et al., 2012). In particular, an excess of visceral adiposity has been shown to be a predictor for the onset of many metabolic related conditions such as insulin resistance, type 2 diabetes, impaired glucose tolerance, dyslipidemia, hypertension and metabolic syndrome, and all of these metabolic conditions are associated with an increased risk of CVD (Li et al., 2012). A change in waist circumference ranging from 1.8 and 4.1 cm has been reported to be clinically relevant in individuals with a waistline between 60 – 135 cm (Verweij et al., 2013). Since waist circumference is a useful indicator of visceral fat, the significant reduction in waist circumference observed in all groups in our study is of potential clinical importance.

Nevertheless, further research that delves into the effect of *C. fimbriata* and *C. sinensis* extract on regional adipose tissue distribution and the relationship between visceral adiposity and the metabolic profile is needed. Clinical trials of a longer duration may also identify significant changes in body composition. The focus should be on the different abdominal fat

areas including abdominal visceral fat, abdominal subcutaneous fat, and total abdominal fat via CT-scan, as it is of importance considering the conflicting results in the current literature.

The non-significant treatment effect of *C. fimbriata* extract on waist circumference is inconsistent with our previous human trial and other studies. Kuriyan et al (2007) identified a significant decline in waistline in the experimental group at the end of the 60 day intervention period. The non-significant treatment effect in waist circumference in the *C. sinensis* group is also inconsistent with previous studies following administration of similar formulas of red orange extract (Dallas et al., 2008, Dallas et al., 2014). It has been postulated that reductions in waist circumference may be attributed to the presence of flavonoids in red oranges. Many studies have identified that flavonoids possess lipolytic activity through the inhibition of cAMP-phosphodiesterase and sustaining lipolysis-inducing cAMP levels (Kuppusamy and Das, 1992). Dallas et al (2008) demonstrated that SINETROL (combination of flavonoids and guarana) is a potent inhibitor of cAMP-phosphodiesterase activity.

Furthermore, the mechanisms involved in the reduction of fat accumulation following Moro orange supplementation in previous studies may involve the anthocyanins that are abundant in the blood orange. The effect of Moro orange juice (combination of *C. sinensis* + Navelina, a blond orange) administration on fat accumulation was investigated in high fat fed mice by Titta et al (2010). It was observed that Moro anthocyanin-rich juice prevented body weight gain, fat development and inhibited high-fat diet induced obesity in mice (Titta et al., 2010). The authors stated that Moro juice appears to directly target the ability of adipocytes to accumulate fat. It was then revealed in a study by Tsuda et al (2006), that Moro juice is

capable of effectively counteracting the consequences of high fat diet-induced obesity on adipocyte gene expression. Tsuda et al (2006) found that the gene expression profile in human adipocytes treated with anthocyanins significantly changed the expression of adipocytokines including the up-regulation of adiponectin and the down regulation of PAI-1 and IL-6. Additionally, lipid metabolism related genes comprising of uncoupling protein 2, acylCoA oxidase 1 and perilipin were found to be up-regulated following anthocyanin treatment. The authors concluded that the up-regulation of these genes may limit excess lipid accumulation in adipocytes (Tsuda et al., 2006).

Another key association between an accumulation of fat mass and obesity related complications is an increase in oxidative status and a low-grade inflammatory state (Dallas et al., 2014). Several studies have established the link between a high level of inflammatory biomarkers such as CRP and obesity related comorbidities including CVD, diabetes, hypertension and atherosclerosis in overweight and obese individuals (de Ferranti and Mozaffarian, 2008, Nguyen et al., 2009). In our study, there was a significant time effect for the inflammatory biomarker, CRP. However this also appears to be due to the hypocaloric diet prescription followed. A preview study by Dallas et al (2014), showed a significant decrease in inflammatory biomarkers including CRP and fibrinogen levels following supplementation of Sinetrol-XPur, a combination extract of red orange, grape fruit, and orange. Furthermore, another study also demonstrated the anti-inflammatory activities associated with red orange juice intake (Buscemi et al., 2012). Buscemi et al (2012) found significant reductions in CRP concentration following red orange juice intake in a cross over study design. C-reactive protein concentrations were reported to be <2 mg/L in Buscemi's study, which is associated with a 30 % reduction in the risk of cardiovascular events (Ridker

et al., 2005). Other inflammatory cytokines including IL-6 and TNF-α were also reduced in the study by Buscemi et al (2014). Therefore the reduction in inflammatory cytokines chiefly CRP may lessen inflammatory signalling with potentially positive effects on atherosclerotic progression (Wilson et al., 2007). The anti-inflammatory action of red oranges observed in human trials is in agreement with in vitro studies (Cardile et al., 2010). The anti-inflammatory action of red oranges (in vitro) is mediated by a decrease in the expression of intracellular adhesion molecule 1, monocyte chemoattractant protein-1, and IL-8 in normal human keratinocites stimulated with c-interferon and histamine (Cardile et al., 2010).

To the best of our knowledge, this is the first human trial to measure the effect of *C. fimbriata* extract on the inflammatory biomarker, CRP. It was hypothesised that *C. fimbriata* may cause a decrease in CRP levels due to the presence of phenolic compounds namely flavonoids or sterols/triterpenes. It has been postulated that the anti-inflammatory activity of *C. fimbriata* could be achieved through inhibition of cyclooxygenase, which is involved in the synthesis of inflammatory prostaglandins as it has been demonstrated that *C. fimbriata* contains flavonoids which are known to possess anti-inflammatory properties (Saivasanthi et al., 2011).

Chronic dyslipidemia is a major cause of atherosclerosis, a vascular condition affecting blood circulation in the central, coronary and peripheral arteries. CVD risk can be measured via blood lipid profile markers including LDL cholesterol, HDL cholesterol and atherogenic index. A significant reduction in these captured markers of CVD risk in humans may constitute as a way of measuring the efficacy of anti-dyslipidemic therapies (Manickam et al.,

2011). In the present study, there was a significant reduction in total cholesterol and LDL cholesterol although unexpectedly HDL cholesterol also reduced and triglycerides increased in all groups. The decline in HDL cholesterol observed in all groups may be explained by the significant reduction in mono and polyunsaturated fats. Moreover, the significant reduction in total cholesterol is an imperative and modifiable risk factor for CHD. A sustained decrease in total cholesterol of 1 % is linked with a 2-3 % decline in the incidence of CHD (Law et al., 1994). It should also be noted that the significantly lower concentration of total cholesterol and triglyceride level observed in the placebo group was unexpected. The study participants were not randomised according to blood lipid profile, which may be considered as a limitation of the study.

It was hypothesised that *C. fimbriata* extract would improve blood lipid profile in humans, due to the positive results observed in animal models. The therapeutic potential of *C. fimbriata* in dyslipidemia has been investigated in several animal models of diet induced obesity (Somnath et al., 2012, Jagtap et al., 2013, Ambadasu et al., 2013a, Ambadasu et al., 2013b, Kamalakkannan et al., 2010). The authors of the previous animal study conducted by Kamalakkannan et al (2010) attributed the lipid lowering and anti-atherosclerotic effects of *C. fimbriata* to the conferred antioxidant (Ansari et al., 2005) and anti-inflammatory properties of the botanical extract (Zakaria et al., 2001). The cholesterol reducing action has also been reported in other *Caralluma* species including *C. umbellate* and *C. adcendens* (Adnan et al., 2014).

The non-significant treatment effect of *C. sinensis* on dyslipidemia is inconsistent with previous work. The efficacy of berry derived-anthocyanin supplementation (from bilberry [*Vaccinium myrtillus*] and black currant [*Ribes nigrum*]) on dyslipidemia has been evaluated in a previous human trial (Qin et al., 2009), which showed that in dyslipidemic patients, anthocyanin supplementation produced favourable effects on lipoproteins. Qin et al (2009) suggests that administration of anthocyanins may lead to an improvement in lipoprotein profile via lowering plasma LDL-cholesterol and increasing HDL-cholesterol levels in part through the inhibition of cholesteryl ester transfer protein (CETP) target. In support of the observations by Qin et al (2009), this mechanism of lipid lowering action of anthocyanins via inhibition of CETP activity has been reported in animal studies (Lam et al., 2008).

Furthermore, other animal studies have also observed the anti-dyslipidemic activities of red orange extracts. The study conducted by Salamone et al (2012) reported a significant improvement in dyslipidemia following oral administration of Moro orange juice in high fat fed mice (Salamone et al., 2012). The results of Salamone et al (2012) in reversing the metabolic abnormalities associated with a high fat fed diet are in agreement with other animal models of obesity following administration of anthocyanin enriched plant extracts (Galvano et al., 2007). For example, Kwon et al (2007) identified that anthocyanin extracted from black soybean resulted in marketable improvements in dyslipidemia and a decrease in central obesity in high fat fed rodents (Kwon et al., 2007).

The effect of *C. fimbriata* and/or *C. sinensis* on glycaemic control was also evaluated via measurement of fasting blood glucose levels and plasma insulin concentration. No change in blood glucose levels or plasma insulin concentration was observed in all treatment groups. The study by Kuriyan et al (2007) and our previous pilot study also failed to identify any

change in fasting blood sugar levels, despite the reported anti-diabetic activity observed in animal models (Jagtap et al., 2013, Somnath et al., 2012), which includes our own animal study. In this case, it can be highlighted that the positive effects observed in animal models may not translate into similar benefits in the human clinical setting. Due to the gross metabolic dysfunction associated with diabetes, clinical trials conducted on non-diabetic and diabetic subjects using well designed randomised placebo controlled protocols are needed. As such, rigorous investigations using cell lines, ex vivo and animal models of diabetes ideally should be conducted first.

The anti-hyperglycaemic activity of the red orange extract has been evaluated in human studies. Qin et al (2009) analysed the glucose concentration of participants via the glucose oxidase method. No significant differences in glucose concentrations were observed between the anthocyanin treated group compared to control (Qin et al., 2009). Buscemi et al (2012) also adopted the glucose oxidase method for measuring glucose concentrations and also did not identify any change in pre-prandial or post-prandial values. While in contrast to the aforementioned studies, Dallas et al (2014) reported a significant decline in blood glucose levels (9.95 ± 1.87 %) in the Sinetrol-XPur group (red orange, grape fruit, and orange extract). However, the authors attributed the improvement in glucose levels to the presence of grape fruit enriched with naringenin and nargingin, which have been shown to reduce insulin resistance in patients with metabolic syndrome (Fujioka et al., 2006). The in vivo anti-hyperglycemic action of naringenin is mediated via the inhibition of intestinal glucose uptake and renal glucose reabsorption (Dallas et al., 2014).

In the current study, there were no significant changes in physiological parameters including systolic and diastolic blood pressure with the exception of heart rate. Our study reported a slight increase in heart rate over time. A clinical human trial conducted by Dallas et al (2014) evaluating the efficacy of Sinetrol-XPur, a combination extract of red orange, grape fruit, and orange also reported a slightly higher heart rate at the end of the study in the Sinetrol-XPur treatment group (+3.32 %), although it should be noted that all values were still within normal limits of 74 to 77 beats/minute for both studies (Dallas et al., 2014). Dallas et al (2014) indicated that the increase in heart rate could be comparable with drinking three cups of coffee daily in relation to the amount of caffeine (19.8 mg/day). It should also be noted that both botanical extracts were generally well tolerated over the intervention period in the current study. Only minor adverse side effects were reported in the experimental groups, which is consistent with previous toxicology studies on *C. fimbriata* and *C. sinensis* extracts. A major advantage of herbal supplements is the relatively low incidence of adverse events that usually relate to a gentle impact on one's metabolism in contrast to highly potent pharmaceutical drugs with reported undesirable adverse effects. For example, Xenical (Orlistat) is an approved drug in Australia for the treatment of obesity, but it is associated with unpleasant side effects, including fatty/ loose stools, increased defecation, flatus with discharge, faecal urgency/ incontinence, uncontrolled oily discharge, nausea/vomiting, abdominal pain and lower serum levels of fat-soluble vitamins compared to the placebo group (Murray et al., 2008).

The dietary intake of participants was also monitored throughout the intervention period of the present study. By controlling dietary intake during the supplementation period, it was deemed helpful in maintaining compliance of the ingestion of the extract. In support of this

intervention method adopted in the present study, many other studies evaluating the efficacy of weight loss aids have used this approach (combination of supplementation and providing dietary modifications) (Celleno et al., 2007, Nagao et al., 2005). According to the food frequency questionnaire data, there was a significant time effect for energy, total fat, saturated fat, polyunsaturated fat, monounsaturated fat, protein, carbohydrate, fibre, sugar, and salt The significant time effects observed from the FFQ results support the explanation that the changes in body composition parameters were achieved by following the hypocaloric diet. However, there was no significant time effect observed for the food diary data. The inconsistencies between the findings of the food diary recordings and FFQ are possibly attributed to the differences in methodologies of reporting dietary intake used (i.e., the FFQ offers an extensive but not thorough list of items of food consumed during a given period of time, while in comparison the food diary depend on participants' ability to recall dietary information). Therefore it is recognized that the present study is limited by inter-individual variability in food intake and the subjective nature of reporting and interpreting. Furthermore, the validity and reliability of the dietary intake findings may be strengthened by analysing biomarkers such as urinary minerals or nitrogen excretion (Rothenberg, 1994).

There are inconsistent findings regarding the effect of *C. fimbriata* extract on food intake regulation in humans. In our previous pilot study, there were also significant improvements in dietary intake in both groups, including a reduction in total fat and saturated fat intake, as well as a notable increase in whole grains, fruits and vegetables over the intervention period. While, Kuriyan et al (2007) found that only *C. fimbriata* extract supplementation significantly reduced refined sugar intake, sweets, cholesterol, and saturated fat intake over time, however the intake of fruits, vegetables and fish remained unchanged.

In contrast, the effect of anthocyanin supplementation on energy and macronutrients in previous studies are not compelling. A previous human trial evaluating the efficacy of anthocyanin ingestion only asked participants to maintain habitual diet and lifestyle during the intervention period, with no significant change observed in mean daily intakes of nutrients reported over the 12-week treatment period (Qin et al., 2009). The study by Dallas et al (2014), controlled dietary intake with the caloric level set at 1800 – 2000kcal/day for women and 2000 – 2500 kcal/day for men. Physical activity recommendations were also provided, where subjects were instructed to participate in 30 minutes of exercise per week (three sessions of 10 minute walks). A diet and exercise questionnaire was also administered to detect any differences among groups, however the results of the questionnaire were unfortunately not stated (Dallas et al., 2014). The study by Buscemi et al (2012) consisted of a randomised, 2 x 2 crossover, placebo-controlled, single blind design, where participants were studied for two periods of 7 ± 1 day (periods 1 & 2), separated by a 3-day interval. During the intervention period, subjects were asked to maintain dietary habits and their exercise routine, however it was requested that the dietary intake of participants to be similar to that of three days preceding baseline measurements and during the last 3-days of periods 1 and 2. A 24-hour food diary recall was also compiled the day before measurements. The authors stated that there were no significant differences observed in total energy and macronutrient intakes in the food diaries provided (Buscemi et al., 2012).

The eating behaviour and assessment of appetite of participants was monitored during the 12-week treatment period. No change in hunger and fullness sensations, satiety quotient for the degree of hunger or the appetite hormones ghrelin and leptin were detected in the treatment groups, which is consistent with the food diary recordings. There were no significant

differences in hunger or fullness sensations in the *C. fimbriata* treated group, which is consistent with our previous pilot human trial and animal study. While in contrast the study by Kuriyan et al (2007) demonstrated that the appetite satiety effect of *C. fimbriata* extract was apparent, with a 7.2 % increase in the feeling of fullness, a 9.5 % reduction in urge to eat and a 19.7 % decline in hunger levels reported in the *C. fimbriata* supplemented group. This finding supports those of other animal models of diet induced obesity on the efficacy of *C. fimbriata* extract (Kamalakkannan et al., 2010). Whereas, to the best of our knowledge, this is the first study that has investigated the effect of red orange extract on appetite in humans, which unfortunately did not show compelling results. Due to the inconsistencies reported in the literature and the present findings on appetite, it is of importance to clarify the therapeutic role of these botanical extracts in the treatment of obesity.

6.7 Conclusion

In conclusion, we observed favourable changes in body composition in overweight and obese adults which may be attributed to dietary modifications. The energy and macronutrient intakes of participants' significantly improved which is in support of the changes in anthropometry reported in all groups. However, supplementation with *C. fimbriata* extract and *C. sinensis* extract did not significantly provide a treatment effect on the major outcome measures in this study. Therefore, due to the inconsistencies in the results identified in the current study and previous work, a definitive conclusion on the effectiveness of *C. fimbriata* extract and/or *C. sinensis* extract for the management of metabolic and cardiovascular disorders cannot be made. Thus, long term rigorous clinical studies are warranted for future research to better clarify the potential metabolic effects of these two botanical extracts in the management of metabolic and cardiovascular conditions for long-term use.

6.8 Study specific acknowledgements:

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CHAPTER SEVEN: GENERAL DISCUSSION AND CONCLUDING REMARKS

7.1 Introduction

The studies accomplished within this thesis provide an insight into the therapeutic use of botanical extracts, chiefly *Caralluma fimbriata* and *Citrus sinensis* (Moro variety) on metabolic disturbances associated with obesity, metabolic syndrome and the potential risk of atherosclerosis in an overweight or obese state. This research identified inconsistencies in the literature regarding the effectiveness of supplementation with *C. fimbriata* extract and/ or *C. sinensis* extract on metabolic and cardiovascular disorders. These findings provide important clinical evidence that raises the question whether recommending these botanical extracts for the therapeutic management of overweight and obesity and associated metabolic and cardiovascular conditions is valid. This chapter has the intention of integrating the studies completed within this thesis and to provide concluding remarks on the overall outcome of the investigation of the effects of *C. fimbriata* and *C. sinensis* extracts on metabolic and cardiovascular disorders.

7.1.1 The effectiveness of C. fimbriata

Investigations into the anti-obesity effects of *C. fimbriata* extract were explored in Chapters four to six. Here we have shown conflicting findings that supplementation with *C. fimbriata* extract leads to significant reductions in waist circumference under overweight and obese conditions as reported in the pilot human clinical trial, however the major human trial and the animal model of diet induced obesity failed to identify significant changes in central adiposity. An accumulation of central adiposity is detrimental to one's health with substantial evidence supporting the notion that excess visceral adipose tissue is linked with the presence

of metabolic abnormalities namely metabolic syndrome (Despres, 2007). It is unclear from these findings that administration of *C. fimbriata* extract may be used as a therapeutic treatment option for individuals with a large waistline (Men >102 cm (40 in); Women >88 cm (>35 in) (Expert Panel on Detection Evaluation and Treatment of Overweight in Adults, 1998). However, in line with our observations in the pilot study, a previous human trial by Kuriyan et al (2007) also identified significant reductions in waist circumference following *C. fimbriata* supplementation compared to the placebo group. In addition, Kamalakkannan et al (2010) demonstrated that in rodents fed a high fat cafeteria diet plus *C. fimbriata* treatment, there were considerable reductions in fat pads including perirenal, epididymal and mesenteric adipose tissue compared to placebo. These studies undertaken within this thesis adds to the growing research in further understanding the efficacy of *C. fimbriata* extract as an anti-obesity agent especially in the treatment of central obesity.

The overall results from this thesis did not meet the hypothesis that supplementation with *C. fimbriata* extract is capable of reducing metabolic disturbances and thus has important clinical relevance for the therapeutic treatment of metabolic syndrome. The findings of this research showed that supplementation with *C. fimbriata* did not significantly elicit beneficial effects on central adiposity, systolic and diastolic blood pressure, lipid profile, glucose tolerance and liver histopathology in a diet-induced obesity rat model. However these findings oppose previous work. For instances, Latha et al (2014) found that following administration of *C. fimbriata* extract in diabetic induced rats that there was significant recovery of the liver compared to the diabetic control group, which showed complete destruction of hepatocytes, degeneration of central vein, fatty degeneration and damaged hepatocytes. In addition, other studies have shown anti-diabetic effects of *C. fimbriata* supplementation. It has been previously found that *C. fimbriata* administration is capable of

preventing hyperglycemia, partial prevention of hyperinsulinemia and may also significantly reduce plasma glucose levels in rodents (Sudhakara et al., 2014). The lipid lowering properties of *C. fimbriata* extract have been observed in previous animal studies which indicated a significant improvement in lipid profile in cafeteria fed rats treated with *C. fimbriata* extract (Kamalakkannan et al., 2010, Ambadasu et al., 2013a, Ambadasu et al., 2013b, Somnath et al., 2012, Jagtap et al., 2013, Latha et al., 2014). The significant decline in CRP levels has not been reported in previous studies, however the anti-inflammatory properties of other *Caralluma* extracts have been described previously (Adnan et al., 2014). Furthermore, the anti-atherosclerotic properties of *C. fimbriata* have been documented in rodent studies (Kamalakkannan et al., 2010).

The metabolic effects of *C. fimbriata* were also investigated in the clinical setting in chapters four and six, with differing findings observed in humans. There were no significant treatment effects reported in the major human clinical trial (chapter six), while only a noteworthy decline in waist circumference was observed in the experimental group in the pilot study (chapter four). However, both human trials failed to identify significant reductions in hunger via the VAS method, therefore suggesting *C. fimbriata* extract may not have appetite suppressing properties. However, it should be noted that a significant decline in energy intake and waist circumference was reported in the study conducted by Kuriyan et al (2007). Kuriyan also identified the appetite suppressing activity of *C. fimbriata* extract through the reduction in hunger and fullness ratings via the VAS method.

From the previous work conducted on the effectiveness of *C. fimbriata* supplementation and the research presented within this thesis, it is evident that there is conflicting evidence regarding the metabolic effects of *C. fimbriata* in the treatment of metabolic and cardiovascular disorders in rodents and humans. There is currently not enough conclusive

evidence to suggest that *C. fimbriata* supplementation is capable of reducing all components of metabolic syndrome in humans, despite the significant findings observed in previous animal studies. Further research is required to validate the potential benefits of *C. fimbriata* in the treatment of obesity comorbidities. Possible future directions for the investigation into the role *C. fimbriata* plays in the treatment of obesity associated pathologies are described in section 8.2.

7.1.2 The effectiveness of C. sinensis

The botanical extract, *C. sinensis* (Moro variety) was investigated alone and in combination with *C. fimbriata* extract for its potential anti-obesity and anti-inflammatory properties (chapter six). The results from this thesis revealed no significant treatment effect of *C. sinensis* supplementation alone or in combination with *C. fimbriata* extract on metabolic and cardiovascular disorders in overweight and obese adults. However these findings are in conflict with previous work. For example, improvements in anthropometric measurements including BMI, waist and hip circumferences following *C. sinensis* treatment have been reported in other studies investigating combination formulas of anthocyanin supplementation in overweight individuals (Dallas et al., 2008, Dallas et al., 2014). Additionally, the anti-inflammatory effects of *C. sinensis* (various anthocyanin formulas) extract have been explored in previous human studies (Dallas et al., 2014, Buscemi et al., 2012). In light of the results from our research and the reported findings of previous studies, it is unclear whether supplementation with *C. sinensis* extract is a useful anti-obesity agent for the management of overweight and obesity and associated comorbidities.

7.2 General conclusions

C. fimbriata extract is an edible succulent plant native to India, with anecdotal evidence of appetite suppressing and anti-obesity properties. In recent years, the beneficial properties of the botanical extract have been explored for its therapeutic role in the treatment of obesity and metabolic disturbances (Kamalakkannan et al., 2010, Kuriyan et al., 2007, Lawrence and Choudhary, 2004). Importantly, the results contained within this thesis provide significant contribution to the literature, highlighting that the evidence supporting *C. fimbriata* extract and or *C. sinensis* extract as anti-obesity agents is weak. However, the dietary modifications to participants' food intake were clinically meaningful, providing significant improvements to body composition parameters. Further research may be required to better clarify the efficacy of these botanical extracts, or it may not even be worthwhile pursuing further. An alternative study design or a larger sample size may be necessary to show significance.

C. sinensis (Moro variety) is a red orange that originated from the Mediterranean regions. The strong red colour of the Moro orange is attributed to the abundance of anthocyanins. Establishing the clinical efficacy of red coloured varieties enriched with anthocyanins such as Moro, Sanuinello and Tarocco as anti-obesity agents has recently been explored in animal models and in the clinical setting (Titta et al., 2010, Dallas et al., 2014). Importantly, our findings presented in chapter six do not support current literature that *C. sinensis* may be useful as a therapeutic strategy for the treatment of central adiposity. The findings of this study importantly merit the consideration of further investigations in the area of metabolic outcomes associated with the use of anthocyanin supplementation.

CHAPTER EIGHT: RECOMMENDATIONS FOR FUTURE RESEARCH

Due to the inconsistent findings reported within this thesis and the current literature, it is recommended that future work in this area may focus on developing larger, long-term well designed clinical trials for determining the efficacy of chronic administration of *C. fimbriata* extract for the treatment of obesity and associated conditions. It is also of interest to further investigate the mechanisms underlying the proposed beneficial properties of the major plant extract (*C. fimbriata*) investigated within this thesis. This chapter will identify gaps in knowledge and attempt to provide recommendations for future research in this area.

8.1 Larger sample size and long-term supplementation of C. fimbriata and Moro orange extracts in humans with metabolic abnormalities

Even though the total number of participants satisfied the minimum number required by power analysis in the present human trials reported within this thesis, the sample size was still relatively small and it is unlikely that it was a true representation of the general overweight and obese adult population, thus limiting the applicability of the results. For instance, the majority of the participants who completed the each study were female. It would be useful for future studies to restrict participation to a more specific sample population and/or recruit a larger sample size.

Currently, the majority of clinical studies on obesity treatment with use of natural weight loss products have been conducted for relatively short-term intervention periods (<12 weeks duration). In addition, there is a lack of studies that include a follow-up phase to identify weight maintenance following treatment cessation. Therefore, long-term therapy of

overweight and obesity is required to demonstrate that weight loss can be achieved and maintained. Larger clinical trials documenting the treatment effect for at least one year are needed to demonstrate the efficacy and safety for long-term use. Currently, there are no human clinical trials that have investigated the effects of chronic administration (one year duration) of *C. fimbriata* extract or *C. sinensis* extract on long term body composition changes in humans. Therefore clinical trials of a longer duration and larger sample sizes are required to verify the beneficial effects of *C. fimbriata* extract and *C. sinensis* supplementation.

Furthermore, the use of placebo controlled clinical trials particularly in long-term studies is often linked with a high dropout rate. To minimise non-adherence to intervention protocols and thus reduce dropout rates investigators may empirically probe subjects adherence during a "run-in period" and thereafter only randomize adherent participants into the trial (Pablos-Mendez et al., 1998). This run-in period protocol could be adopted in future randomised controlled trials on *C. fimbriata* and *C. sinensis* extracts. Furthermore, it would also be of significance to conduct follow-up trials to determine weight reduction maintenance over time. Follow-up weight maintenance trials are yet to be performed following supplementation of *C. fimbriata* and *C. sinensis* extracts.

Studies have shown that subjects who receive concomitant dietary advice and follow an exercise program in addition to phytochemical supplementation show significant additional anti-obesity effects (Hasani-Ranjbar et al., 2009). Long-term weight maintenance is dependent on sustained behavioural changes including dietary modification and maintaining a regular physical activity regime. Accordingly, long-term prevention of relapse of reduced

weight gain following intentional weight loss remains a therapeutic challenge among clinicians and the research community. Adjunctive phytotherapy such as *C. fimbriata* extract or *C. sinensis* extract may facilitate long-term weight maintenance for humans. One potential approach in preventing weight regain is to reduce appetite and promote satiety signals. As it has been reported in previous studies that *C. fimbriata* and *C. sinensis* are capable of suppressing appetite or energy intake, future research could focus on chronic supplementation of these botanical extracts on weight regain following a weight loss program. Another aim for future research could evaluate the long-term effects (greater than one year duration) of *C. fimbriata* extract and *C. sinensis* extract combined with behavioural counselling, appropriate dietary changes and an exercise program (overall wellbeing) on obesity-related cardio-metabolic risk factors.

This future research with the goal of establishing the efficacy of *C. fimbriata* and *C. sinensis* extracts in long-term well designed clinical trials serves to substantiate positive empirical evidence, creating a more robust evidence base for the safe and effective use of both *C. fimbriata* and *C. sinensis* supplementation as a therapeutic option for overweight and obese adults.

8.2 Functional role of C. fimbriata extract in the treatment of metabolic syndrome

Reliable and accurate measurements of body composition are critical in the interpretation and evaluation of findings reported in research studies that consist of a weight loss intervention program. There is also an accumulation of evidence that links body shape and dimensions with health risk (Pouliot et al., 1994, Despres, 2007). Thus, measurement of body

composition particularly regional adiposity is important in detecting small changes in body fat content in response to an intervention. Ideally, the significant findings reported in previous studies of reduced waist circumference observed in the *C. fimbriata* and *C. sinensis* group should be validated with future research that demonstrated changes in visceral fat mass. The measurement of central adiposity could be achieved through the measurement of abdominal visceral fat (AVF), abdominal subcutaneous fat (ASF), and total abdominal fat (TAF) areas via computed tomography (CT)-scan.

There is a close link established between adipose tissue metabolism, differential fat distribution and disorders such as diabetes, obesity and CVD (Bjorntorp, 1987). The animal study presented in this thesis is lacking analysis of some of the specific fat depots that are associated with metabolic risk such as omental, mesenteric, brown adipose tissue and deep subcutaneous adipose tissue (Bjorndal et al., 2011). Therefore, future animal research could focus on the assessment of metabolically active adipose tissue in order to better elucidate the anti-obesity effects of *C. fimbriata* extract.

Furthermore, a deeper investigation into the mechanisms underlying the reduction of abdominal fat may be explored through histological analysis of adipose tissue and the analysis of adipocyte specific gene expression. Therefore future research could investigate the gene expression profile of human adipocytes treated with *C. fimbriata* to identify changes in the regulation of adipocytokines such as adiponectin, PAI-1, TNF-α and leptin. Moreover, lipid metabolism related genes that are involved in transcriptional regulation of lipolysis and lipogenesis such as peroxisome proliferator-activated receptors (PPARs) (Kersten, 2002)

could also be investigated to elucidate the effect of *C. fimbriata* on adipocyte function, in addition to the previous work conducted by Akbarsha et al., (2010).

As previously discussed in chapter two, metabolic syndrome is associated with impaired fibrinolysis, micro-inflammation and oxidative stress, which are considered major risk factors for CVD and type 2 diabetes (Trevisan et al., 1998). Future human research could investigate parameters characterising oxidative status in subjects with metabolic syndrome following chronic *C. fimbriata* extract supplementation, through the analysis of superoxide dismutase, catalase, and arginase activities. Other oxidative markers that could be analysed include lipid peroxidation, myeloperoxidase activity, nitrite, and hydrogen peroxide concentrations in plasma (da Fonseca et al., 2014). The proinflammatory cytokine profile including IL-6, TNF-α and PAI-1 could also be investigated to better understand the role of *C. fimbrata* extract in inflammatory associated conditions.

Previous animal models of diet induced obesity have established the lipid lowering activities of *C. fimbriata* extract (Somnath et al., 2012). In addition, it is evident from the findings of previous studies (Kamalakkannan et al., 2010) that *C. fimbriata* extract supplementation has potential anti-atherosclerotic properties. However the mechanisms by which the plant extract elicits these effects are not fully understood. It is possible that the anti-atherosclerotic activity of *C. fimbriata* is attributed to the flavonoids present in the extract. Hence, future studies that focus on understanding the molecular mechanisms and gene-level effects of the active ingredients including flavonoids are warranted given their reported anti-atherosclerotic properties. In continuation of this investigation, proinflammatory cytokines that often exist in

atherosclerotic lesions and CVD biomarkers such as homocysteine and microalbumin levels, and VCAM-1 and ICAM-1 levels in plasma just to name a few could then be investigated to gain a more comprehensive understanding of the role *C. fimbriata* extract plays in the amelioration of atherosclerosis.

REFERENCES

ABATE, N., GARG, A., PESHOCK, R. M., STRAY-GUNDERSEN, J. & GRUNDY, S. M. 1995. Relationships of generalized and regional adiposity to insulin sensitivity in men. *J Clin Invest,* 96**,** 88-98.

ABBASI, F., BROWN, B. W., JR., LAMENDOLA, C., MCLAUGHLIN, T. & REAVEN, G. M. 2002. Relationship between obesity, insulin resistance, and coronary heart disease risk. *J Am Coll Cardiol,* 40**,** 937-43.

ACADEMY OF NUTRITION AND DIETETICS. 2010. *Adult Weight Management Evidence-Based Nutrition Practice Guideline* [Online]. Available: www.eatright.org.

ACQUAVIVA, R., RUSSO, A., GALVANO, F., GALVANO, G., BARCELLONA, M. L., LI VOLTI, G. & VANELLA, A. 2003. Cyanidin and cyanidin 3-O-beta-D -glucoside as DNA cleavage protectors and antioxidants. *Cell Biol Toxicol,* 19**,** 243-52.

ADNAN, M., JAN, S., MUSSARAT, S., TARIQ, A., BEGUM, S., AFROZ, A. & SHINWARI, Z. K. 2014. A review on ethnobotany, phytochemistry and pharmacology of plant genus Caralluma R. Br. *J Pharm Pharmacol,* 66**,** 1351-68.

AKBARSHA, M., CLAYTON, P., KAMALAKKANNAN, S., RAJENDRAN, R. & VENKATESH, R. 2010. Effect of Caralluma Fimbriata extract on 3T3-L1 pre-adipocyte cell division. *Science Research,* 2**,** 329-336.

ALAIN, C., PON, L., CHAN, C., RICHMOND, W., AND FU P. 1974. Enzymatic Determination of Total Serum Cholesterol. *Clin Chem,* 20**,** 470-475.

ALBERTI, K. G., ECKEL, R. H., GRUNDY, S. M., ZIMMET, P. Z., CLEEMAN, J. I., DONATO, K. A., FRUCHART, J. C., JAMES, W. P., LORIA, C. M., SMITH, S. C., JR., INTERNATIONAL DIABETES FEDERATION TASK FORCE ON, E., PREVENTION, HATIONAL HEART, L., BLOOD, I., AMERICAN HEART, A.,

WORLD HEART, F., INTERNATIONAL ATHEROSCLEROSIS, S. & INTERNATIONAL ASSOCIATION FOR THE STUDY OF, O. 2009. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation,* 120**,** 1640-5.

ALBERTI, K. G., ZIMMET, P. & SHAW, J. 2006. Metabolic syndrome--a new world-wide definition. A Consensus Statement from the International Diabetes Federation. *Diabet Med,* 23**,** 469-80.

ALESSI, M. C., PEIRETTI, F., MORANGE, P., HENRY, M., NALBONE, G. & JUHAN-VAGUE, I. 1997. Production of plasminogen activator inhibitor 1 by human adipose tissue: possible link between visceral fat accumulation and vascular disease. *Diabetes,* 46**,** 860-7.

ALEXANDER, C. M., LANDSMAN, P. B., TEUTSCH, S. M. & HAFFNER, S. M. 2003. NCEP-defined metabolic syndrome, diabetes, and prevalence of coronary heart disease among NHANES III participants age 50 years and older. *Diabetes,* 52**,** 1210-4.

ALVAREZ, G. E., BESKE, S. D., BALLARD, T. P. & DAVY, K. P. 2002. Sympathetic neural activation in visceral obesity. *Circulation,* 106**,** 2533-6.

AMBADASU, B., DANGES, S. V. & WALI, R. S. 2013a. Effect of Caralluma fimbriata extract on appetite, body weight & lipid profile in cafeteria diet-induced obesity in rats. *Int J Pharm Pharm Sci,* 5, 536-539.

AMBADASU, B., DANGES, S. V., WALI, R. S. & WORLIKAR, P. S. 2013b. Effect of Caralluma fimbriata extract on appetite & lipid profile in rats fed with hypercalorie/ cafeteria diet. *Int J Pharm Pharm Sci,* 4, 788-793.

ANDERSON, P. J., CRITCHLEY, J. A., CHAN, J. C., COCKRAM, C. S., LEE, Z. S., THOMAS, G. N. & TOMLINSON, B. 2001. Factor analysis of the metabolic syndrome: obesity vs insulin resistance as the central abnormality. *Int J Obes Relat Metab Disord,* 25**,** 1782-8.

ANSARI, N. M., HOULIHAN, L., HUSSAIN, B. & PIERONI, A. 2005. Antioxidant activity of five vegetables traditionally consumed by South-Asian migrants in Bradford, Yorkshire, UK. *Phytother Res,* 19**,** 907-11.

ARITA, Y., KIHARA, S., OUCHI, N., TAKAHASHI, M., MAEDA, K., MIYAGAWA, J., HOTTA, K., SHIMOMURA, I., NAKAMURA, T., MIYAOKA, K., KURIYAMA, H., NISHIDA, M., YAMASHITA, S., OKUBO, K., MATSUBARA, K., MURAGUCHI, M., OHMOTO, Y., FUNAHASHI, T. & MATSUZAWA, Y. 1999. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun,* 257**,** 79-83.

ASONGALEM, E. A., FOYET, H. S., EKOBO, S., DIMO, T. & KAMTCHOUING, P. 2004. Antiinflammatory, lack of central analgesia and antipyretic properties of Acanthus montanus (Ness) T. Anderson. *J Ethnopharmacol,* 95**,** 63-8.

ASSMANN, G., SCHULTE, H. & VON ECKARDSTEIN, A. 1996a. Hypertriglyceridemia and elevated lipoprotein(a) are risk factors for major coronary events in middle-aged men. *Am J Cardiol,* 77**,** 1179-84.

ASSMANN, G., SCHULTE, H., VON ECKARDSTEIN, A. & HUANG, Y. 1996b. High-density lipoprotein cholesterol as a predictor of coronary heart disease risk. The

PROCAM experience and pathophysiological implications for reverse cholesterol transport. *Atherosclerosis,* 124 Suppl**,** S11-20.

ASTELL, K. J., MATHAI, M. L., MCAINCH, A. J., STATHIS, C. G. & SU, X. Q. 2013a. A pilot study investigating the effect of Caralluma fimbriata extract on the risk factors of metabolic syndrome in overweight and obese subjects: a randomised controlled clinical trial. *Complement Ther Med,* 21**,** 180-9.

ASTELL, K. J., MATHAI, M. L. & SU, X. Q. 2013b. Plant extracts with appetite suppressing properties for body weight control: a systematic review of double blind randomized controlled clinical trials. *Complement Ther Med,* 21**,** 407-16.

ASTELL, K. J., MATHAI, M. L. & SU, X. Q. 2013c. A review on botanical species and chemical compounds with appetite suppressing properties for body weight control. *Plant Foods Hum Nutr,* 68**,** 213-21.

AUSTRALIAN BETTER HEALTH INITIATIVE. 2008. *How do you measure up?* [Online]. Available: http://www.health.gov.au/internet/abhi/publishing.nsf/Content/factsheet-waist-measurement [Accessed 27 September 2010.

AUSTRALIAN BUREAU OF STATISTICS. 2013. Overweight/ Obesity 4125.0 - Gender Indicators, Australia, Jan 2013.

AUSTRALIAN GOVERNMENT 2005. Food for Health; Dietary Guidelines for Australians; A guide to healthy eating. *In:* DEPARTMENT OF HEALTH AND AGEING: NATIONAL HEALTH AND MEDICAL RESEARCH COUNCIL (ed.).

AVOGARO, P., CREPALDI, G 1965. Essential hyperlipidemia, obesity and diabetes. *Diabetologia,* 1**,** 137.

AZIZI, F., SALEHI, P., ETEMADI, A. & ZAHEDI-ASL, S. 2003. Prevalence of metabolic syndrome in an urban population: Tehran Lipid and Glucose Study. *Diabetes Res Clin Pract,* 61**,** 29-37.

BADER, A., BRACA, A., DE TOMMASI, N. & MORELLI, I. 2003. Further constituents from Caralluma negevensis. *Phytochemistry,* 62**,** 1277-81.

BALASURIYA, N. & RUPASINGHE, H. P. 2012. Antihypertensive properties of flavonoid-rich apple peel extract. *Food Chem,* 135**,** 2320-5.

BALKAU, B. & CHARLES, M. A. 1999. Comment on the provisional report from the WHO consultation. European Group for the Study of Insulin Resistance (EGIR). *Diabet Med,* 16**,** 442-3.

BARINAS-MITCHELL, E., CUSHMAN, M., MEILAHN, E. N., TRACY, R. P. & KULLER, L. H. 2001. Serum levels of C-reactive protein are associated with obesity, weight gain, and hormone replacement therapy in healthy postmenopausal women. *Am J Epidemiol,* 153**,** 1094-101.

BASTARD, J. P., JARDEL, C., BRUCKERT, E., BLONDY, P., CAPEAU, J., LAVILLE, M., VIDAL, H. & HAINQUE, B. 2000. Elevated levels of interleukin 6 are reduced in serum and subcutaneous adipose tissue of obese women after weight loss. *J Clin Endocrinol Metab,* 85**,** 3338-42.

BECKMAN, T., SHI, Q. & BILLINGTON, C. 2005. The brain and the biology of obesity. *Minn Med,* 88**,** 58-61.

BELTRAN-SANCHEZ, H., HARHAY, M. O., HARHAY, M. M. & MCELLIGOTT, S. 2013. Prevalence and trends of metabolic syndrome in the adult U.S. population, 1999-2010. *J Am Coll Cardiol,* 62**,** 697-703.

BERCHTOLD, P., JORGENS, V., FINKE, C. & BERGER, M. 1981. Epidemiology of obesity and hypertension. *Int J Obes,* 5 suppl 1**,** 1-7.

BERG, A. H., COMBS, T. P., DU, X., BROWNLEE, M. & SCHERER, P. E. 2001. The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. *Nat Med,* 7**,** 947-53.

BERTHOUD, H. R. 2008. The vagus nerve, food intake and obesity. *Regul Pept,* 149**,** 15-25.

BERTIN, E., NGUYEN, P., GUENOUNOU, M., DURLACH, V., POTRON, G. & LEUTENEGGER, M. 2000. Plasma levels of tumor necrosis factor-alpha (TNF-alpha) are essentially dependent on visceral fat amount in type 2 diabetic patients. *Diabetes Metab,* 26**,** 178-82.

BJORNDAL, B., BURRI, L., STAALESEN, V., SKORVE, J. & BERGE, R. K. 2011. Different adipose depots: their role in the development of metabolic syndrome and mitochondrial response to hypolipidemic agents. *J Obes,* 2011**,** 490650.

BJORNTORP, P. 1987. Adipose tissue distribution, plasma insulin, and cardiovascular disease. *Diabete Metab,* 13**,** 381-5.

BLOOMGARDEN, Z. T. 2002. Obesity, hypertension, and insulin resistance. *Diabetes Care,* 25**,** 2088-97.

BONINA, F. P., LEOTTA, C., SCALIA, G., PUGLIA, C., TROMBETTA, D., TRINGALI, G., ROCCAZZELLO, A. M., RAPISARDA, P. & SAIJA, A. 2002. Evaluation of oxidative stress in diabetic patients after supplementation with a standardised red orange extract. *Diabetes Nutr Metab,* 15**,** 14-9.

BONINA, F. P., PUGLIA, C., CIMINO, F., TROMBETTA, D., TRINGALI, G., ROCCAZZELLO, A. M., INSIRELLO, E., RAPISARDA, P. & SAIJA, A. 2005. Oxidative stress in handball players: effect of supplementation with a red orange extract. *Nutr Res,* 25.

BOSELLO, O. & ZAMBONI, M. 2000. Visceral obesity and metabolic syndrome. *Obes Rev,* 1**,** 47-56.

BOUSTANY, C. M., BHARADWAJ, K., DAUGHERTY, A., BROWN, D. R., RANDALL, D. C. & CASSIS, L. A. 2004. Activation of the systemic and adipose renin-

angiotensin system in rats with diet-induced obesity and hypertension. *Am J Physiol Regul Integr Comp Physiol,* 287**,** R943-9.

BREA, A., MOSQUERA, D., MARTIN, E., ARIZTI, A., CORDERO, J. L. & ROS, E. 2005. Nonalcoholic fatty liver disease is associated with carotid atherosclerosis: a case-control study. *Arterioscler Thromb Vasc Biol,* 25**,** 1045-50.

BREWER, H. B., JR. 1999. Hypertriglyceridemia: changes in the plasma lipoproteins associated with an increased risk of cardiovascular disease. *Am J Cardiol,* 83**,** 3F-12F.

BROUILLARD, R. & CHEMINAT, A. 1988. Flavonoids and plant color. *Prog Clin Biol Res,* 280**,** 93-106.

BUETTNER, R., SCHOLMERICH, J. & BOLLHEIMER, L. C. 2007. High-fat diets: modeling the metabolic disorders of human obesity in rodents. *Obesity (Silver Spring),* 15**,** 798-808.

BUSCEMI, S., ROSAFIO, G., ARCOLEO, G., MATTINA, A., CANINO, B., MONTANA, M., VERGA, S. & RINI, G. 2012. Effects of red orange juice intake on endothelial function and inflammatory markers in adult subjects with increased cardiovascular risk. *Am J Clin Nutr,* 95**,** 1089-95.

CAMERON, A. J., MAGLIANO, D. J., ZIMMET, P. Z., WELBORN, T. & SHAW, J. E. 2007. The metabolic syndrome in Australia: prevalence using four definitions. *Diabetes Res Clin Pract* 77**,** 471-8.

CAMERON, A. J., SHAW, J. E. & ZIMMET, P. Z. 2004. The metabolic syndrome: prevalence in worldwide populations. *Endocrinol Metab Clin North Am,* 33**,** 351-75, table of contents.

CARDILE, V., FRASCA, G., RIZZA, L., RAPISARDA, P. & BONINA, F. 2010. Antiinflammatory effects of a red orange extract in human keratinocytes treated with interferon-gamma and histamine. *Phytother Res,* 24**,** 414-8.

CAREY, D. G., JENKINS, A. B., CAMPBELL, L. V., FREUND, J. & CHISHOLM, D. J. 1996. Abdominal fat and insulin resistance in normal and overweight women: Direct measurements reveal a strong relationship in subjects at both low and high risk of NIDDM. *Diabetes,* 45**,** 633-8.

CARO, J. F., SINHA, M. K., KOLACZYNSKI, J. W., ZHANG, P. L. & CONSIDINE, R. V. 1996. Leptin: the tale of an obesity gene. *Diabetes,* 45**,** 1455-62.

CARR, D. B., UTZSCHNEIDER, K. M., HULL, R. L., KODAMA, K., RETZLAFF, B. M., BRUNZELL, J. D., SHOFER, J. B., FISH, B. E., KNOPP, R. H. & KAHN, S. E. 2004. Intra-abdominal fat is a major determinant of the National Cholesterol Education Program Adult Treatment Panel III criteria for the metabolic syndrome. *Diabetes,* 53**,** 2087-94.

CELLENO, L., TOLAINI, M. V., D'AMORE, A., PERRICONE, N. V. & PREUSS, H. G. 2007. A Dietary supplement containing standardized Phaseolus vulgaris extract influences body composition of overweight men and women. *Int J Med Sci,* 4**,** 45-52.

CHAPMAN, M. J. 2006. Therapeutic elevation of HDL-cholesterol to prevent atherosclerosis and coronary heart disease. *Pharmacol Ther,* 111**,** 893-908.

CHEHREI, A., SADRNIA, S., KESHTELI, A. H., DANESHMAND, M. A. & REZAEI, J. 2007. Correlation of dyslipidemia with waist to height ratio, waist circumference, and body mass index in Iranian adults. *Asia Pac J Clin Nutr,* 16**,** 248-53.

CIOFFI, G., SANOGO, R., VASSALLO, A., DAL PIAZ, F., AUTORE, G., MARZOCCO, S. & DE TOMMASI, N. 2006. Pregnane glycosides from Leptadenia pyrotechnica. *J Nat Prod* 69**,** 625-35.

CLEARFIELD, M. B. 2005. C-reactive protein: a new risk assessment tool for cardiovascular disease. *J Am Osteopath Assoc,* 105**,** 409-16.

CNOP, M., HAVEL, P. J., UTZSCHNEIDER, K. M., CARR, D. B., SINHA, M. K., BOYKO, E. J., RETZLAFF, B. M., KNOPP, R. H., BRUNZELL, J. D. & KAHN, S. E. 2003. Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma lipoproteins: evidence for independent roles of age and sex. *Diabetologia,* 46**,** 459-69.

CNOP, M., LANDCHILD, M. J., VIDAL, J., HAVEL, P. J., KNOWLES, N. G., CARR, D. R., WANG, F., HULL, R. L., BOYKO, E. J., RETZLAFF, B. M., WALDEN, C. E., KNOPP, R. H. & KAHN, S. E. 2002. The concurrent accumulation of intra-abdominal and subcutaneous fat explains the association between insulin resistance and plasma leptin concentrations : distinct metabolic effects of two fat compartments. *Diabetes,* 51**,** 1005-15.

COLEMAN, N. S., MARCIANI, L., BLACKSHAW, E., WRIGHT, J., PARKER, M., YANO, T., YAMAZAKI, S., CHAN, P. Q., WILDE, K., GOWLAND, P. A., PERKINS, A. C. & SPILLER, R. C. 2003. Effect of a novel 5-HT3 receptor agonist MKC-733 on upper gastrointestinal motility in humans. *Aliment Pharmacol Ther,* 18**,** 1039-48.

COMBS, T. P., BERG, A. H., OBICI, S., SCHERER, P. E. & ROSSETTI, L. 2001. Endogenous glucose production is inhibited by the adipose-derived protein Acrp30. *J Clin Invest,* 108**,** 1875-81.

CONE, R. D., COWLEY, M. A., BUTLER, A. A., FAN, W., MARKS, D. L. & LOW, M. J. 2001. The arcuate nucleus as a conduit for diverse signals relevant to energy homeostasis. *Int J Obes Relat Metab Disord,* 25 Suppl 5**,** S63-7.

CONSIDINE, R. V., SINHA, M. K., HEIMAN, M. L., KRIAUCIUNAS, A., STEPHENS, T. W., NYCE, M. R., OHANNESIAN, J. P., MARCO, C. C., MCKEE, L. J., BAUER,

T. L. & ET AL. 1996. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med,* 334**,** 292-5.

COOK, T., RUTISHAUSER, I., SEELIG, M., 2001. Comparable data on food and nutrient intake and physical measurements from the 1983, 1985 and 1995 national nutrition surveys. *In:* AUSTRALIAN FOOD AND NUTRITION MONITORING UNIT, A. G. (ed.). Canberra.

COTRONEO, P., RUSSO, M., CIUNI, M., RECUPERO, G. 2006. Quantitative real-time reverse transcriptase-PCR profiling of anthocyanin biosynthetic genes during orange fruit ripening *J Am Soc Hort Sci,* 131.

CRAIG, C. L., MARSHALL, A. L., SJOSTROM, M., BAUMAN, A. E., BOOTH, M. L., AINSWORTH, B. E., PRATT, M., EKELUND, U., YNGVE, A., SALLIS, J. F. & OJA, P. 2003. International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc,* 35**,** 1381-95.

CUBEDDU, L. X., BONISCH, H., GOTHERT, M., MOLDERINGS, G., RACKE, K., RAMADORI, G., MILLER, K. J. & SCHWORER, H. 2000. Effects of metformin on intestinal 5-hydroxytryptamine (5-HT) release and on 5-HT3 receptors. *Naunyn Schmiedebergs Arch Pharmacol,* 361**,** 85-91.

CUMMINGS, D. E. & OVERDUIN, J. 2007. Gastrointestinal regulation of food intake. *J Clin Invest,* 117**,** 13-23.

DA FONSECA, L. J., NUNES-SOUZA, V., GUEDES GDA, S., SCHETTINO-SILVA, G., MOTA-GOMES, M. A. & RABELO, L. A. 2014. Oxidative status imbalance in patients with metabolic syndrome: role of the myeloperoxidase/hydrogen peroxide axis. *Oxid Med Cell Longev,* 2014**,** 898501.

DALLAS, C., GERBI, A., ELBEZ, Y., CAILLARD, P., ZAMARIA, N. & CLOAREC, M. 2014. Clinical Study to Assess the Efficacy and Safety of a Citrus Polyphenolic

Extract of Red Orange, Grapefruit, and Orange (Sinetrol-XPur) on Weight Management and Metabolic Parameters in Healthy Overweight Individuals. *Phytother Res,* 28**,** 212–218.

DALLAS, C., GERBI, A., TENCA, G., JUCHAUX, F. & BERNARD, F. X. 2008. Lipolytic effect of a polyphenolic citrus dry extract of red orange, grapefruit, orange (SINETROL) in human body fat adipocytes. Mechanism of action by inhibition of cAMP-phosphodiesterase (PDE). *Phytomedicine,* 15**,** 783-92.

DALTON, M., CAMERON, A. J., ZIMMET, P. Z., SHAW, J. E., JOLLEY, D., DUNSTAN, D. W. & WELBORN, T. A. 2003. Waist circumference, waist-hip ratio and body mass index and their correlation with cardiovascular disease risk factors in Australian adults. *J Intern Med,* 254**,** 555-63.

DANDONA, P., ALJADA, A., CHAUDHURI, A., MOHANTY, P. & GARG, R. 2005. Metabolic syndrome: a comprehensive perspective based on interactions between obesity, diabetes, and inflammation. *Circulation,* 111**,** 1448-54.

DE FERRANTI, S. & MOZAFFARIAN, D. 2008. The perfect storm: obesity, adipocyte dysfunction, and metabolic consequences. *Clin Chem,* 54**,** 945-55.

DE LEO, M., DE TOMMASI, N., SANOGO, R., AUTORE, G., MARZOCCO, S., PIZZA, C., MORELLI, I. & BRACA, A. 2005. New pregnane glycosides from Caralluma dalzielii. *Steroids,* 70**,** 573-85.

DE LUIS ROMAN, D., DE LA FUENTE, R. A., SAGRADO, M. G., IZAOLA, O. & VICENTE, R. C. 2006. Leptin receptor Lys656Asn polymorphism is associated with decreased leptin response and weight loss secondary to a lifestyle modification in obese patients. *Arch Med Res,* 37**,** 854-9.

DEFRONZO, R. A. 2006. Is insulin resistance atherogenic? Possible mechanisms. *Atheroscler,* Suppl, 7**,** 11-5.

DESPRES, J. P. 2007. Cardiovascular disease under the influence of excess visceral fat. *Crit Pathw Cardiol,* 6**,** 51-9.

DIXON, J. B., BHATHAL, P. S. & O'BRIEN, P. E. 2006. Weight loss and non-alcoholic fatty liver disease: falls in gamma-glutamyl transferase concentrations are associated with histologic improvement. *Obes Surg,* 16**,** 1278-86.

DONATUCCI, D. A., LIENER, I. E. & GROSS, C. J. 1987. Binding of navy bean (Phaseolus vulgaris) lectin to the intestinal cells of the rat and its effect on the absorption of glucose. *J Nutr,* 117**,** 2154-60.

DUTT, H. C., SINGH, S., AVULA, B., KHAN, I. A. & BEDI, Y. S. 2012. Pharmacological review of Caralluma R.Br. with special reference to appetite suppression and anti-obesity. *J Med Food,* 15**,** 108-19.

ECKEL, R. H., GRUNDY, S. M. & ZIMMET, P. Z. 2005. The metabolic syndrome. *Lancet,* 365**,** 1415-28.

EIKELIS, N., SCHLAICH, M., AGGARWAL, A., KAYE, D. & ESLER, M. 2003. Interactions between leptin and the human sympathetic nervous system. *Hypertension,* 41**,** 1072-9.

EINHORN, D., REAVEN, G. M., COBIN, R. H., FORD, E., GANDA, O. P., HANDELSMAN, Y., HELLMAN, R., JELLINGER, P. S., KENDALL, D., KRAUSS, R. M., NEUFELD, N. D., PETAK, S. M., RODBARD, H. W., SEIBEL, J. A., SMITH, D. A. & WILSON, P. W. 2003. American College of Endocrinology position statement on the insulin resistance syndrome. *Endocr Pract,* 9**,** 237-52.

EXPERT PANEL ON DETECTION EVALUATION AND TREATMENT OF OVERWEIGHT IN ADULTS 1998. Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults: executive summary.

Expert Panel on the Identification, Evaluation, and Treatment of Overweight in Adults. *Am J Clin Nutr,* 68**,** 899-917.

FABRICANT, D. S. & FARNSWORTH, N. R. 2001. The value of plants used in traditional medicine for drug discovery. *Environ Health Perspect,* 109 Suppl 1**,** 69-75.

FERRANNINI, E., BUZZIGOLI, G., BONADONNA, R., GIORICO, M. A., OLEGGINI, M., GRAZIADEI, L., PEDRINELLI, R., BRANDI, L. & BEVILACQUA, S. 1987. Insulin resistance in essential hypertension. *N Engl J Med,* 317**,** 350-7.

FERRANNINI, E., HAFFNER, S. M., MITCHELL, B. D. & STERN, M. P. 1991. Hyperinsulinaemia: the key feature of a cardiovascular and metabolic syndrome. *Diabetologia,* 34**,** 416-22.

FERRE, P. & FOUFELLE, F. 2007. SREBP-1c transcription factor and lipid homeostasis: clinical perspective. *Horm Res,* 68**,** 72-82.

FERRI, C., PITTONI, V., PICCOLI, A., LAURENTI, O., CASSONE, M. R., BELLINI, C., PROPERZI, G., VALESINI, G., DE MATTIA, G. & SANTUCCI, A. 1995. Insulin stimulates endothelin-1 secretion from human endothelial cells and modulates its circulating levels in vivo. *J Clin Endocrinol Metab,* 80**,** 829-35.

FESTA, A., D'AGOSTINO, R., JR., HOWARD, G., MYKKANEN, L., TRACY, R. P. & HAFFNER, S. M. 2000. Chronic subclinical inflammation as part of the insulin resistance syndrome: the Insulin Resistance Atherosclerosis Study (IRAS). *Circulation,* 102**,** 42-7.

FESUS, G., DUBROVSKA, G., GORZELNIAK, K., KLUGE, R., HUANG, Y., LUFT, F. C. & GOLLASCH, M. 2007. Adiponectin is a novel humoral vasodilator. *Cardiovasc Res,* 75**,** 719-27.

FOLCH, J., LEES, M. & SLOANE STANLEY, G. H. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem,* 226**,** 497-509.

FONTANA, L., EAGON, J. C., TRUJILLO, M. E., SCHERER, P. E. & KLEIN, S. 2007. Visceral fat adipokine secretion is associated with systemic inflammation in obese humans. *Diabetes,* 56**,** 1010-3.

FORD, E. S., GILES, W. H. & DIETZ, W. H. 2002. Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. *JAMA,* 287**,** 356-9.

FOSSATI, P., PRENCIPE L. 1982. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clin Chem,* 28**,** 2077-80.

FOX, C. S., MASSARO, J. M., HOFFMANN, U., POU, K. M., MAUROVICH-HORVAT, P., LIU, C. Y., VASAN, R. S., MURABITO, J. M., MEIGS, J. B., CUPPLES, L. A., D'AGOSTINO, R. B., SR. & O'DONNELL, C. J. 2007. Abdominal visceral and subcutaneous adipose tissue compartments: association with metabolic risk factors in the Framingham Heart Study. *Circulation,* 116**,** 39-48.

FRIEDEWALD, W. T., LEVY, R. I. & FREDRICKSON, D. S. 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem,* 18**,** 499-502.

FRUEBIS, J., TSAO, T. S., JAVORSCHI, S., EBBETS-REED, D., ERICKSON, M. R., YEN, F. T., BIHAIN, B. E. & LODISH, H. F. 2001. Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. *Proc Natl Acad Sci U S A,* 98**,** 2005-10.

FUJIMOTO, W. Y., ABBATE, S. L., KAHN, S. E., HOKANSON, J. E. & BRUNZELL, J. D. 1994. The visceral adiposity syndrome in Japanese-American men. *Obes Res,* 2**,** 364-71.

FUJIOKA, K., GREENWAY, F., SHEARD, J. & YING, Y. 2006. The effects of grapefruit on weight and insulin resistance: relationship to the metabolic syndrome. *J Med Food,* 9**,** 49-54.

FUJIOKA, S., MATSUZAWA, Y., TOKUNAGA, K. & TARUI, S. 1987. Contribution of intra-abdominal fat accumulation to the impairment of glucose and lipid metabolism in human obesity. *Metabolism,* 36**,** 54-9.

GALVANO, F., LA FAUCI, L., VITAGLIONE, P., FOGLIANO, V., VANELLA, L. & FELGINES, C. 2007. Bioavailability, antioxidant and biological properties of the natural free-radical scavengers cyanidin and related glycosides. *Ann Ist Super Sanita,* 43**,** 382-93.

GARDINER, J. V., KONG, W. M., WARD, H., MURPHY, K. G., DHILLO, W. S. & BLOOM, S. R. 2005. AAV mediated expression of anti-sense neuropeptide Y cRNA in the arcuate nucleus of rats results in decreased weight gain and food intake. *Biochem Biophys Res Commun,* 327**,** 1088-93.

GARRISON, R. J., KANNEL, W. B., STOKES, J., 3RD & CASTELLI, W. P. 1987. Incidence and precursors of hypertension in young adults: the Framingham Offspring Study. *Prev Med,* 16**,** 235-51.

GILES GG. & IRELAND PD. 1996. *Dietary Questionnaire for Epidemiological Studies (Version 2),* Melbourne, The Cancer Council Victoria.

GILTAY, E. J., ELBERS, J. M., GOOREN, L. J., EMEIS, J. J., KOOISTRA, T., ASSCHEMAN, H. & STEHOUWER, C. D. 1998. Visceral fat accumulation is an important determinant of PAI-1 levels in young, nonobese men and women:

modulation by cross-sex hormone administration. *Arterioscler Thromb Vas Biol,* 18**,** 1716-22.

GINSBERG, H. N. 2000. Insulin resistance and cardiovascular disease. *J Clin Invest,* 106**,** 453-8.

GOLDEN, S. H., FOLSOM, A. R., CORESH, J., SHARRETT, A. R., SZKLO, M. & BRANCATI, F. 2002. Risk factor groupings related to insulin resistance and their synergistic effects on subclinical atherosclerosis: the atherosclerosis risk in communities study. *Diabetes,* 51**,** 3069-76.

GOODFRIEND, T. L. & CALHOUN, D. A. 2004. Resistant hypertension, obesity, sleep apnea, and aldosterone: theory and therapy. *Hypertension,* 43**,** 518-24.

GRAF, B. L., RASKIN, I., CEFALU, W. T. & RIBNICKY, D. M. 2010. Plant-derived therapeutics for the treatment of metabolic syndrome. *Curr Opin Investig Drugs,* 11**,** 1107-15.

GRANT, G., DORWARD, P. M. & PUSZTAI, A. 1993. Pancreatic enlargement is evident in rats fed diets containing raw soybeans (Glycine max) or cowpeas (Vigna unguiculata) for 800 days but not in those fed diets based on kidney beans (Phaseolus vulgaris) or lupinseed (Lupinus angustifolius). *J Nutr,* 123**,** 2207-15.

GRANT, R. W. & MEIGS, J. B. 2005. Management of the metabolic syndrome. *Panminerva Medica* 47**,** 219-28.

GREEN, S. M., DELARGY, H. J., JOANES, D. & BLUNDELL, J. E. 1997. A satiety quotient: a formulation to assess the satiating effect of food. *Appetite,* 29**,** 291-304.

GRIFFIN, M. E., MARCUCCI, M. J., CLINE, G. W., BELL, K., BARUCCI, N., LEE, D., GOODYEAR, L. J., KRAEGEN, E. W., WHITE, M. F. & SHULMAN, G. I. 1999. Free fatty acid-induced insulin resistance is associated with activation of protein kinase C theta and alterations in the insulin signaling cascade. *Diabetes,* 48**,** 1270-4.

GRUNDY, S. M., BREWER, H. B., JR., CLEEMAN, J. I., SMITH, S. C., JR. & LENFANT, C. 2004. Definition of metabolic syndrome: report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. *Arterioscler Thromb Vasc Biol,* 24**,** e13-8.

GRUNDY, S. M., CLEEMAN, J. I., DANIELS, S. R., DONATO, K. A., ECKEL, R. H., FRANKLIN, B. A., GORDON, D. J., KRAUSS, R. M., SAVAGE, P. J., SMITH, S. C., JR., SPERTUS, J. A. & COSTA, F. 2005. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation,* 112**,** 2735-52.

GUO, H., LING, W., WANG, Q., LIU, C., HU, Y. & XIA, M. 2008. Cyanidin 3-glucoside protects 3T3-L1 adipocytes against H2O2- or TNF-alpha-induced insulin resistance by inhibiting c-Jun NH2-terminal kinase activation. *Biochem Pharmacol,* 75**,** 1393-401.

HAFFNER, S. M., STERN, M. P., HAZUDA, H. P., PUGH, J. & PATTERSON, J. K. 1987. Do upper-body and centralized adiposity measure different aspects of regional body-fat distribution? Relationship to non-insulin-dependent diabetes mellitus, lipids, and lipoproteins. *Diabetes,* 36**,** 43-51.

HAFFNER, S. M., VALDEZ, R. A., HAZUDA, H. P., MITCHELL, B. D., MORALES, P. A. & STERN, M. P. 1992. Prospective analysis of the insulin-resistance syndrome (syndrome X). *Diabetes,* 41**,** 715-22.

HALL, J. E. 1997. Mechanisms of abnormal renal sodium handling in obesity hypertension. *Am J Hypertens,* 10**,** 49S-55S.

HANLEY, A. J., WILLIAMS, K., FESTA, A., WAGENKNECHT, L. E., D'AGOSTINO, R. B., JR. & HAFFNER, S. M. 2005. Liver markers and development of the metabolic syndrome: the insulin resistance atherosclerosis study. *Diabetes,* 54**,** 3140-7.

HANLEY, A. J., WILLIAMS, K., FESTA, A., WAGENKNECHT, L. E., D'AGOSTINO, R. B., JR., KEMPF, J., ZINMAN, B. & HAFFNER, S. M. 2004. Elevations in markers of liver injury and risk of type 2 diabetes: the insulin resistance atherosclerosis study. *Diabetes,* 53**,** 2623-32.

HASANI-RANJBAR, S., JOUYANDEH, Z. & ABDOLLAHI, M. 2013. A systematic review of anti-obesity medicinal plants - an update. *J Diabetes Metab Disord,* 12**,** 28.

HASANI-RANJBAR, S., NAYEBI, N., LARIJANI, B. & ABDOLLAHI, M. 2009. A systematic review of the efficacy and safety of herbal medicines used in the treatment of obesity. *World J Gastroenterol,* 15**,** 3073-85.

HASLAM, D. W. & JAMES, W. P. 2005. Obesity. *Lancet,* 366**,** 1197-209.

HAVEL, P. J., KASIM-KARAKAS, S., MUELLER, W., JOHNSON, P. R., GINGERICH, R. L. & STERN, J. S. 1996. Relationship of plasma leptin to plasma insulin and adiposity in normal weight and overweight women: effects of dietary fat content and sustained weight loss. *J Clin Endocrinol Metab,* 81**,** 4406-13.

HAYASHI, T., BOYKO, E. J., LEONETTI, D. L., MCNEELY, M. J., NEWELL-MORRIS, L., KAHN, S. E. & FUJIMOTO, W. Y. 2003. Visceral adiposity and the risk of impaired glucose tolerance: a prospective study among Japanese Americans. *Diabetes Care,* 26**,** 650-5.

HEILBRONN, L., SMITH, S. R. & 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. *Int J Obes Relat Metab Disord,* 28 Suppl 4**,** S12-21.

HEINI, A. F., LARA-CASTRO, C., KIRK, K. A., CONSIDINE, R. V., CARO, J. F. & WEINSIER, R. L. 1998. Association of leptin and hunger-satiety ratings in obese women. *Int J Obes Relat Metab Disord,* 22**,** 1084-7.

HO, S. C., CHEN, Y. M., WOO, J. L., LEUNG, S. S., LAM, T. H. & JANUS, E. D. 2001. Association between simple anthropometric indices and cardiovascular risk factors. *Int J Obes Relat Metab Disord,* 25**,** 1689-97.

HOKANSON, J. E. & AUSTIN, M. A. 1996. Plasma triglyceride level is a risk factor for cardiovascular disease independent of high-density lipoprotein cholesterol level: a meta-analysis of population-based prospective studies. *J Cardiovasc Risk,* 3**,** 213-9.

HOTAMISLIGIL, G. S., ARNER, P., CARO, J. F., ATKINSON, R. L. & SPIEGELMAN, B. M. 1995. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. *J Clin Invest,* 95**,** 2409-15.

HOTAMISLIGIL, G. S., PERALDI, P., BUDAVARI, A., ELLIS, R., WHITE, M. F. & SPIEGELMAN, B. M. 1996. IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance. *Science,* 271**,** 665-8.

HOTAMISLIGIL, G. S., SHARGILL, N. S. & SPIEGELMAN, B. M. 1993. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science,* 259**,** 87-91.

HOTTA, K., FUNAHASHI, T., ARITA, Y., TAKAHASHI, M., MATSUDA, M., OKAMOTO, Y., IWAHASHI, H., KURIYAMA, H., OUCHI, N., MAEDA, K., NISHIDA, M., KIHARA, S., SAKAI, N., NAKAJIMA, T., HASEGAWA, K., MURAGUCHI, M., OHMOTO, Y., NAKAMURA, T., YAMASHITA, S., HANAFUSA, T. & MATSUZAWA, Y. 2000. Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. *Arterioscler Thromb Vasc Biol,* 20**,** 1595-9.

HOU, D. X., KAI, K., LI, J. J., LIN, S., TERAHARA, N., WAKAMATSU, M., FUJII, M., YOUNG, M. R. & COLBURN, N. 2004. Anthocyanidins inhibit activator protein 1

activity and cell transformation: structure-activity relationship and molecular mechanisms. *Carcinogenesis,* 25**,** 29-36.

HOWARD, B. V., ROBBINS, D. C., SIEVERS, M. L., LEE, E. T., RHOADES, D., DEVEREUX, R. B., COWAN, L. D., GRAY, R. S., WELTY, T. K., GO, O. T. & HOWARD, W. J. 2000. LDL cholesterol as a strong predictor of coronary heart disease in diabetic individuals with insulin resistance and low LDL: The Strong Heart Study. *Arterioscler Thromb Vasc Biol,* 20**,** 830-5.

HSIAO, P. J., KUO, K. K., SHIN, S. J., YANG, Y. H., LIN, W. Y., YANG, J. F., CHIU, C. C., CHUANG, W. L., TSAI, T. R. & YU, M. L. 2007. Significant correlations between severe fatty liver and risk factors for metabolic syndrome. *J Gastroenterol Hepatol,* 22**,** 2118-23.

HUETTEMAN, D. A. & BOGIE, H. 2009. Direct blood pressure monitoring in laboratory rodents via implantable radio telemetry. *Methods Mol Biol,* 573**,** 57-73.

HULSEY, M. G., PLESS, C. M., WHITE, B. D. & MARTIN, R. J. 1995. ICV administration of anti-NPY antisense oligonucleotide: effects on feeding behavior, body weight, peptide content and peptide release. *Regul Pept,* 59**,** 207-14.

HUNTER, D. J., SAMPSON, L., STAMPFER, M. J., COLDITZ, G. A., ROSNER, B. & WILLETT, W. C. 1988. Variability in portion sizes of commonly consumed foods among a population of women in the United States. *Am J Epidemiol,* 127**,** 1240-9.

IBRAHIM, M. M. 2010. Subcutaneous and visceral adipose tissue: structural and functional differences. *Obes Rev,* 11**,** 11-8.

ISHIZAKA, N., ISHIZAKA, Y., TODA, E., NAGAI, R. & YAMAKADO, M. 2005. Association between serum uric acid, metabolic syndrome, and carotid atherosclerosis in Japanese individuals. *Arterioscler Thromb Vasc Biol,* 25**,** 1038-44.

ISOMAA, B., ALMGREN, P., TUOMI, T., FORSEN, B., LAHTI, K., NISSEN, M., TASKINEN, M. R. & GROOP, L. 2001. Cardiovascular morbidity and mortality associated with the metabolic syndrome. *Diabetes Care,* 24**,** 683-9.

IZAWA, S., OKADA, M., MATSUI, H., HORITA, Y. A. 1997. A new direct method for measuring HDL-cholesterol which does not produce any biased values. *Int J Med Pharm Sci* 37**,** 1385-1388.

JAGTAP, S., SHIDORE, P. P. & DANGE, S. V. 2013. Evaluation of antihyperglycemic and lipid lowering activity of Caralluma fimbriata in diabetes induced rats. *Am J PharmTech Res* 3.

JAYAPRAKASAM, B., OLSON, L. K., SCHUTZKI, R. E., TAI, M. H. & NAIR, M. G. 2006. Amelioration of obesity and glucose intolerance in high-fat-fed C57BL/6 mice by anthocyanins and ursolic acid in Cornelian cherry (Cornus mas). *J Agric Food Chem,* 54**,** 243-8.

JENA, B. S., JAYAPRAKASHA, G. K., SINGH, R. P. & SAKARIAH, K. K. 2002. Chemistry and biochemistry of (-)-hydroxycitric acid from Garcinia. *J Agric Food Chem,* 50**,** 10-22.

JUHAN-VAGUE, I., VAGUE, P., ALESSI, M. C., BADIER, C., VALADIER, J., AILLAUD, M. F. & ATLAN, C. 1987. Relationships between plasma insulin triglyceride, body mass index, and plasminogen activator inhibitor 1. *Diabete Metab,* 13**,** 331-6.

KAMALAKKANNAN, S., RAJENDRAN, R., VENKATESH, R. V., CLAYTON, P. & AKBARSHA, M. A. 2010. Antiobesogenic and Antiatherosclerotic Properties of Caralluma fimbriata Extract. *J Nutr Metab* 2010**,** 285301.

KANAI, H., TOKUNAGA, K., FUJIOKA, S., YAMASHITA, S., KAMEDA-TAKEMURA, K. K. & MATSUZAWA, Y. 1996. Decrease in intra-abdominal visceral fat may reduce blood pressure in obese hypertensive women. *Hypertension,* 27**,** 125-9.

KANELLIS, J. & KANG, D. H. 2005. Uric acid as a mediator of endothelial dysfunction, inflammation, and vascular disease. *Semin Nephrol,* 25**,** 39-42.

KAPLAN, N. M. 1989. The deadly quartet. Upper-body obesity, glucose intolerance, hypertriglyceridemia, and hypertension. *Arch Intern Med,* 149**,** 1514-20.

KATSUKI, A., SUMIDA, Y., MURASHIMA, S., MURATA, K., TAKARADA, Y., ITO, K., FUJII, M., TSUCHIHASHI, K., GOTO, H., NAKATANI, K. & YANO, Y. 1998. Serum levels of tumor necrosis factor-alpha are increased in obese patients with noninsulin-dependent diabetes mellitus. *J Clin Endocrinol Metab,* 83**,** 859-62.

KATSUKI, A., SUMIDA, Y., URAKAWA, H., GABAZZA, E. C., MURASHIMA, S., MARUYAMA, N., MORIOKA, K., NAKATANI, K., YANO, Y. & ADACHI, Y. 2003. Increased visceral fat and serum levels of triglyceride are associated with insulin resistance in Japanese metabolically obese, normal weight subjects with normal glucose tolerance. *Diabetes Care,* 26**,** 2341-4.

KAUR, J. 2014. A Comprehensive Review on Metabolic Syndrome. *Cardiol Res Pract,* 2014**,** 943162.

KAWAMOTO, R., TOMITA, H., OKA, Y. & OHTSUKA, N. 2006. Relationship between serum uric acid concentration, metabolic syndrome and carotid atherosclerosis. *Intern Med,* 45**,** 605-14.

KEIM, N. L., STERN, J. S. & HAVEL, P. J. 1998. Relation between circulating leptin concentrations and appetite during a prolonged, moderate energy deficit in women. *Am J Clin Nutr,* 68**,** 794-801.

KERN, P. A., DI GREGORIO, G. B., LU, T., RASSOULI, N. & RANGANATHAN, G. 2003. Adiponectin expression from human adipose tissue: relation to obesity, insulin resistance, and tumor necrosis factor-alpha expression. *Diabetes,* 52**,** 1779-85.

KERN, P. A., RANGANATHAN, S., LI, C., WOOD, L. & RANGANATHAN, G. 2001. Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. *Am J Physiol Endocrinol Metab,* 280**,** E745-51.

KERSTEN, S. 2002. Peroxisome proliferator activated receptors and obesity. *Eur J Pharmacol,* 440**,** 223-34.

KISSEBAH, A. H., VYDELINGUM, N., MURRAY, R., EVANS, D. J., HARTZ, A. J., KALKHOFF, R. K. & ADAMS, P. W. 1982. Relation of body fat distribution to metabolic complications of obesity. *J Clin Endocrinol Metab,* 54**,** 254-60.

KNUDSON, J. D., DINCER, U. D., ZHANG, C., SWAFFORD, A. N., JR., KOSHIDA, R., PICCHI, A., FOCARDI, M., DICK, G. M. & TUNE, J. D. 2005. Leptin receptors are expressed in coronary arteries, and hyperleptinemia causes significant coronary endothelial dysfunction. *Am J Physiol Heart Circ Physiol,* 289**,** H48-56.

KOMARNYTSKY, S., ESPOSITO, D., RATHINASABAPATHY, T., POULEV, A. & RASKIN, I. 2013. Effects of pregnane glycosides on food intake depend on stimulation of the melanocortin pathway and BDNF in an animal model. *J Agric Food Chem,* 61**,** 1841-9.

KUNERT, O., RAO, V. G., BABU, G. S., SUJATHA, P., SIVAGAMY, M., ANURADHA, S., RAO, B. V., KUMAR, B. R., ALEX, R. M., SCHUHLY, W., KUHNELT, D., RAO, G. V. & RAO, A. V. 2008. Pregnane glycosides from Caralluma adscendens var. fimbriata. *Chem Biodivers,* 5**,** 239-50.

KUPPUSAMY, U. R. & DAS, N. P. 1992. Effects of flavonoids on cyclic AMP phosphodiesterase and lipid mobilization in rat adipocytes. *Biochem Pharmacol,* 44**,** 1307-15.

KURIYAN, R., RAJ, T., SRINIVAS, S. K., VAZ, M., RAJENDRAN, R. & KURPAD, A. V. 2007. Effect of Caralluma fimbriata extract on appetite, food intake and anthropometry in adult Indian men and women. *Appetite,* 48**,** 338-44.

KURPAD, A., KHAN, K., CALDER, A. G., COPPACK, S., FRAYN, K., MACDONALD, I. & ELIA, M. 1994. Effect of noradrenaline on glycerol turnover and lipolysis in the whole body and subcutaneous adipose tissue in humans in vivo. *Clin Sci (Lond),* 86**,** 177-84.

KWON, E. K., LEE, D. Y., LEE, H., KIM, D. O., BAEK, N. I., KIM, Y. E. & KIM, H. Y. 2010. Flavonoids from the buds of Rosa damascena inhibit the activity of 3-hydroxy-3-methylglutaryl-coenzyme a reductase and angiotensin I-converting enzyme. *J Agric Food Chem,* 58**,** 882-6.

KWON, S. H., AHN, I. S., KIM, S. O., KONG, C. S., CHUNG, H. Y., DO, M. S. & PARK, K. Y. 2007. Anti-obesity and hypolipidemic effects of black soybean anthocyanins. *J Med Food,* 10**,** 552-6.

KYLIN, E. 1923. Studien uber das Hypertonie-Hyperglyka 'mie-Hyperurika' miesyndrom. *Zentralbl Inn Med,* 44**,** 105-127.

LACKLAND, D. T., ORCHARD, T. J., KEIL, J. E., SAUNDERS, D. E., JR., WHEELER, F. C., ADAMS-CAMPBELL, L. L., MCDONALD, R. H. & KNAPP, R. G. 1992. Are race differences in the prevalence of hypertension explained by body mass and fat distribution? A survey in a biracial population. *Int J Epidemiol,* 21**,** 236-45.

LAM, C. K., ZHANG, Z., YU, H., TSANG, S. Y., HUANG, Y. & CHEN, Z. Y. 2008. Apple polyphenols inhibit plasma CETP activity and reduce the ratio of non-HDL to HDL cholesterol. *Mol Nutr Food Res,* 52**,** 950-8.

LANDIN, K., STIGENDAL, L., ERIKSSON, E., KROTKIEWSKI, M., RISBERG, B., TENGBORN, L. & SMITH, U. 1990. Abdominal obesity is associated with an impaired fibrinolytic activity and elevated plasminogen activator inhibitor-1. *Metabolism,* 39**,** 1044-8.

LATHA, S., RAJARAM, K. & SURESH KUMAR, P. 2014. Hepatoprotective and antidiabetic effect of methanol extract of Caralluma fimbriata in streptatozocin induced diabetic albino rats. *Int J Pharm Pharm Sci,* 6, 665-668.

LAW, M. R., WALD, N. J. & THOMPSON, S. G. 1994. By how much and how quickly does reduction in serum cholesterol concentration lower risk of ischaemic heart disease? *BMJ,* 308**,** 367-72.

LAWRENCE, R. & CHOUDHARY, S. Caralluma fimbriata in the treatment of obesity. The proceedings of the 12th Annual World Congress of Anti-Aging Medicine, 2004 Las Vegas, Nev, USA.

LEMIEUX, I., PASCOT, A., COUILLARD, C., LAMARCHE, B., TCHERNOF, A., ALMERAS, N., BERGERON, J., GAUDET, D., TREMBLAY, G., PRUD'HOMME, D., NADEAU, A. & DESPRES, J. P. 2000. Hypertriglyceridemic waist: A marker of the atherogenic metabolic triad (hyperinsulinemia; hyperapolipoprotein B; small, dense LDL) in men? *Circulation,* 102**,** 179-84.

LI, X., KATASHIMA, M., YASUMASU, T. & LI, K. J. 2012. Visceral fat area, waist circumference and metabolic risk factors in abdominally obese Chinese adults. *Biomed Environ Sci,* 25**,** 141-8.

LO SCALZO, R., IANNOCCARI, T., SUMMA, C., MORELLI, R., RAPISARDA P. 2004. Effect of thermal treatments on antoxidant and antiradical activity of blood orange. *Food Chem,* 85.

LOIZZO, M. R., SAID, A., TUNDIS, R., RASHED, K., STATTI, G. A., HUFNER, A. & MENICHINI, F. 2007. Inhibition of angiotensin converting enzyme (ACE) by flavonoids isolated from Ailanthus excelsa (Roxb) (Simaroubaceae). *Phytother Res,* 21**,** 32-6.

LOWENSTEIN, J. M. 1971. Effect of (-)-hydroxycitrate on fatty acid synthesis by rat liver in vivo. *J Biol Chem,* 246**,** 629-32.

LUTSEY, P. L., STEFFEN, L. M. & STEVENS, J. 2008. Dietary intake and the development of the metabolic syndrome: the Atherosclerosis Risk in Communities study. *Circulation,* 117**,** 754-61.

MACLEAN, D. B. & LUO, L. G. 2004. Increased ATP content/production in the hypothalamus may be a signal for energy-sensing of satiety: studies of the anorectic mechanism of a plant steroidal glycoside. *Brain Res,* 1020**,** 1-11.

MAFFEI, M., HALAAS, J., RAVUSSIN, E., PRATLEY, R. E., LEE, G. H., ZHANG, Y., FEI, H., KIM, S., LALLONE, R., RANGANATHAN, S. & ET AL. 1995. Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. *Nat Med,* 1**,** 1155-61.

MALINOW, M. R. 1995. Plasma homocyst(e)ine and arterial occlusive diseases: a mini-review. *Clin Chem,* 41**,** 173-6.

MANICKAM, P., RATHOD, A., PANAICH, S., HARI, P., VEERANNA, V., BADHEKA, A., JACOB, S. & AFONSO, L. 2011. Comparative prognostic utility of conventional and novel lipid parameters for cardiovascular disease risk prediction: do novel lipid parameters offer an advantage? *J Clin Lipidol,* 5**,** 82-90.

MARCHESINI, G., BRIZI, M., BIANCHI, G., TOMASSETTI, S., BUGIANESI, E., LENZI, M., MCCULLOUGH, A. J., NATALE, S., FORLANI, G. & MELCHIONDA, N. 2001. Nonalcoholic fatty liver disease: a feature of the metabolic syndrome. *Diabetes,* 50**,** 1844-50.

MARQUEZ, F., BABIO, N., BULLO, M. & SALAS-SALVADO, J. 2012. Evaluation of the safety and efficacy of hydroxycitric acid or Garcinia cambogia extracts in humans. *Crit Rev Food Sci Nutr,* 52**,** 585-94.

MATSUBARA, M., MARUOKA, S. & KATAYOSE, S. 2002a. Decreased plasma adiponectin concentrations in women with dyslipidemia. *J Clin Endocrinol Metab,* 87**,** 2764-9.

MATSUBARA, M., MARUOKA, S. & KATAYOSE, S. 2002b. Inverse relationship between plasma adiponectin and leptin concentrations in normal-weight and obese women. *Eur J Endocrinol,* 147**,** 173-80.

MATSUZAWA, Y. 1997. Pathophysiology and molecular mechanisms of visceral fat syndrome: the Japanese experience. *Diabetes Metab Rev,* 13**,** 3-13.

MATTES, R. D. & BORMANN, L. 2000. Effects of (-)-hydroxycitric acid on appetitive variables. *Physiol Behav* 71**,** 87-94.

MAURY, E. & BRICHARD, S. M. 2010. Adipokine dysregulation, adipose tissue inflammation and metabolic syndrome. *Mol Cell Endocrinol,* 314**,** 1-16.

MAVRI, A., STEGNAR, M., KREBS, M., SENTOCNIK, J. T., GEIGER, M. & BINDER, B. R. 1999. Impact of adipose tissue on plasma plasminogen activator inhibitor-1 in dieting obese women. *Arterioscler Thromb Vasc Biol,* 19**,** 1582-7.

MCCULLOUGH, A. J. 2004. The clinical features, diagnosis and natural history of nonalcoholic fatty liver disease. *Clin Liver Dis,* 8**,** 521-33, viii.

MCGILL, J. B., SCHNEIDER, D. J., ARFKEN, C. L., LUCORE, C. L. & SOBEL, B. E. 1994. Factors responsible for impaired fibrinolysis in obese subjects and NIDDM patients. *Diabetes,* 43**,** 104-9.

MCGOWAN, M., ARTISS, JD., STRANDBERGH, DR., ZAK B. 1983. A peroxidase-coupled method for the colorimetric determination of serum triglycerides. *Clin Chem,* 29**,** 538-42.

MEIGS, J. B., WILSON, P. W., NATHAN, D. M., D'AGOSTINO, R. B., SR., WILLIAMS, K. & HAFFNER, S. M. 2003. Prevalence and characteristics of the metabolic syndrome in the San Antonio Heart and Framingham Offspring Studies. *Diabetes,* 52**,** 2160-7.

MENDIZABAL, Y., LLORENS, S. & NAVA, E. 2013. Hypertension in metabolic syndrome: vascular pathophysiology. *Int J Hypertens,* 2013**,** 230868.

MISRA, A. & VIKRAM, N. K. 2003. Clinical and pathophysiological consequences of abdominal adiposity and abdominal adipose tissue depots. *Nutrition,* 19**,** 457-66.

MODAN, M., HALKIN, H., ALMOG, S., LUSKY, A., ESHKOL, A., SHEFI, M., SHITRIT, A. & FUCHS, Z. 1985. Hyperinsulinemia. A link between hypertension obesity and glucose intolerance. *J Clin Invest,* 75**,** 809-17.

MOMO, C. E., OBEN, J. E., TAZOO, D. & DONGO, E. 2006. Antidiabetic and hypolipidaemic effects of a methanol/methylene-chloride extract of Laportea ovalifolia (Urticaceae), measured in rats with alloxan-induced diabetes. *Ann Trop Med Parasitol,* 100**,** 69-74.

MONTAGUE, C. T., FAROOQI, I. S., WHITEHEAD, J. P., SOOS, M. A., RAU, H., WAREHAM, N. J., SEWTER, C. P., DIGBY, J. E., MOHAMMED, S. N., HURST, J. A., CHEETHAM, C. H., EARLEY, A. R., BARNETT, A. H., PRINS, J. B. &

O'RAHILLY, S. 1997. Congenital leptin deficiency is associated with severe early-onset obesity in humans. *Nature,* 387**,** 903-8.

MONTALCINI, T., GORGONE, G., FEDERICO, D., CERAVOLO, R., EMANUELE, V., SESTI, G., PERTICONE, F. & PUJIA, A. 2005. Association of LDL cholesterol with carotid atherosclerosis in menopausal women affected by the metabolic syndrome. *Nutr Metab Cardiovasc Dis,* 15**,** 368-72.

MONTEIRO, R. & AZEVEDO, I. 2010. Chronic inflammation in obesity and the metabolic syndrome. *Mediators Inflamm,* 2010.

MONZON, J. R., BASILE, R., HENEGHAN, S., UDUPI, V. & GREEN, A. 2002. Lipolysis in adipocytes isolated from deep and superficial subcutaneous adipose tissue. *Obes Res,* 10**,** 266-9.

MULLEN, K. L., PRITCHARD, J., RITCHIE, I., SNOOK, L. A., CHABOWSKI, A., BONEN, A., WRIGHT, D. & DYCK, D. J. 2009. Adiponectin resistance precedes the accumulation of skeletal muscle lipids and insulin resistance in high-fat-fed rats. *Am J Physiol Regul Integr Comp Physiol,* 296**,** R243-51.

MURRAY, C. D., LE ROUX, C. W., EMMANUEL, A. V., HALKET, J. M., PRZYBOROWSKA, A. M., KAMM, M. A. & MURRAY-LYON, I. M. 2008. The effect of Khat (Catha edulis) as an appetite suppressant is independent of ghrelin and PYY secretion. *Appetite,* 51**,** 747-50.

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**,** 122-9.

NATIONAL HEALTH AND MEDICAL RESEARCH COUNCIL. 2013. Clinical practice guidelines for the management of overweight and obesity in adults, adolescents and children in Australia.,. *In:* DEPARTMENT OF HEALTH.

NGUYEN, X. M., LANE, J., SMITH, B. R. & NGUYEN, N. T. 2009. Changes in inflammatory biomarkers across weight classes in a representative US population: a link between obesity and inflammation. *J Gastrointest Surg,* 13**,** 1205-12.

NHMRC. 2003. Clinical practice guidelines for the management of overweight and obesity in adults. *In:* NATIONAL HEALTH AND MEDICAL RESEARCH COUNCIL. Canberra.

NICKLAS, B. J., ROGUS, E. M., COLMAN, E. G. & GOLDBERG, A. P. 1996. Visceral adiposity, increased adipocyte lipolysis, and metabolic dysfunction in obese postmenopausal women. *Am J Physiol,* 270**,** E72-8.

NIEUWDORP, M., STROES, E. S., MEIJERS, J. C. & BULLER, H. 2005. Hypercoagulability in the metabolic syndrome. *Curr Opin Pharmacol,* 5**,** 155-9.

NIEVES, D. J., CNOP, M., RETZLAFF, B., WALDEN, C. E., BRUNZELL, J. D., KNOPP, R. H. & KAHN, S. E. 2003. The atherogenic lipoprotein profile associated with obesity and insulin resistance is largely attributable to intra-abdominal fat. *Diabetes,* 52**,** 172-9.

O'LEARY, D. H. & POLAK, J. F. 2002. Intima-media thickness: a tool for atherosclerosis imaging and event prediction. *Am J Cardiol,* 90**,** 18L-21L.

ODENDAAL, A. Y., DESHMUKH, N. S., MARX, T. K., SCHAUSS, A. G., ENDRES, J. R. & CLEWELL, A. E. 2013a. Safety Assessment of a Hydroethanolic Extract of Caralluma fimbriata. *Int J Toxicol,* 32**,** 385-394.

ODENDAAL, A. Y., DESHMUKH, N. S., MARX, T. K., SCHAUSS, A. G., ENDRES, J. R. & CLEWELL, A. E. 2013b. Safety assessment of a hydroethanolic extract of Caralluma fimbriata. *Int J Toxicol,* 32**,** 385-94.

OHIA, S. E., AWE, S. O., LEDAY, A. M., OPERE, C. A. & BAGCHI, D. 2001. Effect of hydroxycitric acid on serotonin release from isolated rat brain cortex. *Res Commun Mol Pathol Pharmacol,* 109**,** 210-6.

OHIA, S. E., OPERE, C. A., LEDAY, A. M., BAGCHI, M., BAGCHI, D. & STOHS, S. J. 2002. Safety and mechanism of appetite suppression by a novel hydroxycitric acid extract (HCA-SX). *Mol Cell Biochem,* 238**,** 89-103.

OJEDA, D., JIMENEZ-FERRER, E., ZAMILPA, A., HERRERA-ARELLANO, A., TORTORIELLO, J. & ALVAREZ, L. 2010. Inhibition of angiotensin convertin enzyme (ACE) activity by the anthocyanins delphinidin- and cyanidin-3-O-sambubiosides from Hibiscus sabdariffa. *J Ethnopharmacol,* 127**,** 7-10.

OLIVER, F. J., DE LA RUBIA, G., FEENER, E. P., LEE, M. E., LOEKEN, M. R., SHIBA, T., QUERTERMOUS, T. & KING, G. L. 1991. Stimulation of endothelin-1 gene expression by insulin in endothelial cells. *J Biol Chem,* 266**,** 23251-6.

OSTLUND, R. E., JR., YANG, J. W., KLEIN, S. & GINGERICH, R. 1996. Relation between plasma leptin concentration and body fat, gender, diet, age, and metabolic covariates. *J Clin Endocrinol Metab,* 81**,** 3909-13.

OUCHI, N., KIHARA, S., ARITA, Y., MAEDA, K., KURIYAMA, H., OKAMOTO, Y., HOTTA, K., NISHIDA, M., TAKAHASHI, M., NAKAMURA, T., YAMASHITA, S., FUNAHASHI, T. & MATSUZAWA, Y. 1999. Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. *Circulation,* 100**,** 2473-6.

PABLOS-MENDEZ, A., BARR, R. G. & SHEA, S. 1998. Run-in periods in randomized trials: implications for the application of results in clinical practice. *JAMA,* 279**,** 222-5.

PAGOTTO, U., VANUZZO, D., VICENNATI, V. & PASQUALI, R. 2008. [Pharmacological therapy of obesity]. *G Ital Cardiol (Rome),* 9**,** 83S-93S.

PALANIAPPAN, L., CARNETHON, M. & FORTMANN, S. P. 2003. Association between microalbuminuria and the metabolic syndrome: NHANES III. *Am J Hypertens,* 16**,** 952-8.

PAOLETTI, R., BOLEGO, C., POLI, A. & CIGNARELLA, A. 2006. Metabolic syndrome, inflammation and atherosclerosis. *Vasc Health Risk Manag,* 2**,** 145-52.

PARAMASIVAM, R. & SHANMUGASUNDARAM, K. 2013. Potent antihyperglycaemic activity of Calocybe indica in streptozotocin induced diabetic rats antihyperglycemic activity of Calocybe indica. *Int J Pharm Pharm Sci,* 5**,** 512-515.

PARKER, B. A., STURM, K., MACINTOSH, C. G., FEINLE, C., HOROWITZ, M. & CHAPMAN, I. M. 2004. Relation between food intake and visual analogue scale ratings of appetite and other sensations in healthy older and young subjects. *Eur J Clin Nutr,* 58**,** 212-8.

PASCOT, A., DESPRES, J. P., LEMIEUX, I., BERGERON, J., NADEAU, A., PRUD'HOMME, D., TREMBLAY, A. & LEMIEUX, S. 2000. Contribution of visceral obesity to the deterioration of the metabolic risk profile in men with impaired glucose tolerance. *Diabetologia,* 43**,** 1126-35.

PASCOT, A., LEMIEUX, I., PRUD'HOMME, D., TREMBLAY, A., NADEAU, A., COUILLARD, C., BERGERON, J., LAMARCHE, B. & DESPRES, J. P. 2001. Reduced HDL particle size as an additional feature of the atherogenic dyslipidemia of abdominal obesity. *J Lipid Res,* 42**,** 2007-14.

PELLATI, F., BENVENUTI, S., MELEGARI, M. & FIRENZUOLI, F. 2002. Determination of adrenergic agonists from extracts and herbal products of Citrus aurantium L. var. amara by LC. *J Pharm Biomed Anal,* 29**,** 1113-9.

PERSSON, I. A., PERSSON, K. & ANDERSSON, R. G. 2009. Effect of Vaccinium myrtillus and its polyphenols on angiotensin-converting enzyme activity in human endothelial cells. *J Agric Food Chem,* 57**,** 4626-9.

PETERSEN, K. F. & SHULMAN, G. I. 2002a. Cellular mechanism of insulin resistance in skeletal muscle. *J R Soc Med,* 95 Suppl 42**,** 8-13.

PETERSEN, K. F. & SHULMAN, G. I. 2002b. Pathogenesis of skeletal muscle insulin resistance in type 2 diabetes mellitus. *Am J Cardiol,* 90**,** 11G-18G.

PLAZA, A., PERRONE, A., BALESTRIERI, M. L., FELICE, F., BALESTRIERI, C., HAMED, A. I., PIZZA, C. & PIACENTE, S. 2005. New unusual pregnane glycosides with antiproliferative activity from Solenostemma argel. *Steroids,* 70**,** 594-603.

POIRIER, P. & DESPRES, J. P. 2003. Waist circumference, visceral obesity, and cardiovascular risk. *J Cardiopulm Rehabil,* 23**,** 161-9.

POSTORINO, E. 1999. I flavanoni dei succhi di arancia italiani. *Essenze Deriv Agrum*.

POULIOT, M. C., DESPRES, J. P., LEMIEUX, S., MOORJANI, S., BOUCHARD, C., TREMBLAY, A., NADEAU, A. & LUPIEN, P. J. 1994. Waist circumference and abdominal sagittal diameter: best simple anthropometric indexes of abdominal visceral adipose tissue accumulation and related cardiovascular risk in men and women. *Am J Cardiol,* 73**,** 460-8.

PRADHAN, A. D., MANSON, J. E., RIFAI, N., BURING, J. E. & RIDKER, P. M. 2001. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *JAMA,* 286**,** 327-34.

PRATLEY, R. E., HAGBERG, J. M., DENGEL, D. R., ROGUS, E. M., MULLER, D. C. & GOLDBERG, A. P. 2000. Aerobic exercise training-induced reductions in abdominal fat and glucose-stimulated insulin responses in middle-aged and older men. *J Am Geriatr Soc* 48**,** 1055-61.

PREUSS, H. G., BAGCHI, D., BAGCHI, M., RAO, C. V., DEY, D. K. & SATYANARAYANA, S. 2004a. Effects of a natural extract of (-)-hydroxycitric acid (HCA-SX) and a combination of HCA-SX plus niacin-bound chromium and Gymnema sylvestre extract on weight loss. *Diabetes, Obesity and Metabolism* 6**,** 171-80.

PREUSS, H. G., RAO, C. V., GARIS, R., BRAMBLE, J. D., OHIA, S. E., BAGCHI, M. & BAGCHI, D. 2004b. An overview of the safety and efficacy of a novel, natural(-)-hydroxycitric acid extract (HCA-SX) for weight management. *J Med,* 35**,** 33-48.

PUSZTAI, A., GRANT, G., DUGUID, T., BROWN, D. S., PEUMANS, W. J., VAN DAMME, E. J. & BARDOCZ, S. 1995. Inhibition of starch digestion by alpha-amylase inhibitor reduces the efficiency of utilization of dietary proteins and lipids and retards the growth of rats. *J Nutr,* 125**,** 1554-62.

QIN, Y., XIA, M., MA, J., HAO, Y., LIU, J., MOU, H., CAO, L. & LING, W. 2009. Anthocyanin supplementation improves serum LDL- and HDL-cholesterol concentrations associated with the inhibition of cholesteryl ester transfer protein in dyslipidemic subjects. *Am J Clin Nutr,* 90**,** 485-92.

RAJENDRAN, R., VYAWAHARE, N. S., KHANDARE, R. A., AMBIKAR, D. B. & SANNAPURI, V. D. 2008. Evaluation of nootropic activity of Slimaluma, an enriched phytochemical composition of Caralluma fimbriata in mice. *Planta Medica,* 74**,** 953-954.

RANDLE, P. J., GARLAND, P. B., HALES, C. N. & NEWSHOLME, E. A. 1963. The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. *Lancet,* 1**,** 785-9.

RAPISARDA, P. 1996. Sample preparation for vitamin C analysis of pigmented orange juices. *Ital J Food Sci,* 46.

RAPISARDA, P., & INTRIGLIOLO, F. 2001. Anthocyanins in blood orange: Compostion and biological activity. *Pandalai SG,* 5.

RAPISARDA, P., CAROLLO, G., FALLICO, B., TOMASELLI, F. & MACCARONE, E. 1998. Hydroxycinnamic Acids as Markers of Italian Blood Orange Juices. *J Agric Food Chem,* 46**,** 464-470.

REAVEN, G. M. 1988. Banting lecture 1988. Role of insulin resistance in human disease. *Diabetes,* 37**,** 1595-607.

RENAUD, S. & DE LORGERIL, M. 1992. Wine, alcohol, platelets, and the French paradox for coronary heart disease. *Lancet,* 339**,** 1523-6.

RENDELL, M., HULTHEN, U. L., TORNQUIST, C., GROOP, L. & MATTIASSON, I. 2001. Relationship between abdominal fat compartments and glucose and lipid metabolism in early postmenopausal women. *J Clin Endocrinol Metab,* 86**,** 744-9.

RIBEIRO-FILHO, F. F., FARIA, A. N., KOHLMANN, N. E., ZANELLA, M. T. & FERREIRA, S. R. 2003. Two-hour insulin determination improves the ability of abdominal fat measurement to identify risk for the metabolic syndrome. *Diabetes Care,* 26**,** 1725-30.

RIDKER, P. M. 2003. Clinical application of C-reactive protein for cardiovascular disease detection and prevention. *Circulation,* 107**,** 363-9.

RIDKER, P. M., CANNON, C. P., MORROW, D., RIFAI, N., ROSE, L. M., MCCABE, C. H., PFEFFER, M. A., BRAUNWALD, E., PRAVASTATIN OR ATORVASTATIN, E. & INFECTION THERAPY-THROMBOLYSIS IN MYOCARDIAL INFARCTION, I. 2005. C-reactive protein levels and outcomes after statin therapy. *N Engl J Med,* 352**,** 20-8.

RILEY, P., O'DONOHUE, J. & CROOK, M. 2007. A growing burden: the pathogenesis, investigation and management of non-alcoholic fatty liver disease. *J Clin Pathol,* 60**,** 1384-91.

ROCCHINI, A. P., MOOREHEAD, C., DEREMER, S., GOODFRIEND, T. L. & BALL, D. L. 1990. Hyperinsulinemia and the aldosterone and pressor responses to angiotensin II. *Hypertension,* 15**,** 861-6.

ROCHA, P. M., BARATA, J. T., TEIXEIRA, P. J., ROSS, R. & SARDINHA, L. B. 2008. Independent and opposite associations of hip and waist circumference with metabolic syndrome components and with inflammatory and atherothrombotic risk factors in overweight and obese women. *Metabolism,* 57**,** 1315-22.

ROESCHLAU, P., BERNT, E., GRUBER W. 1974. Enzymatic determination of total cholesterol in serum. *Z Klin Chem Klin Biochem,* 12**,** 226.

ROSENBAUM, M., HIRSCH, J., GALLAGHER, D. A. & LEIBEL, R. L. 2008. Long-term persistence of adaptive thermogenesis in subjects who have maintained a reduced body weight. *Am J Clin Nutr,* 88**,** 906-12.

ROSS, M. & PAWLINA, W. 2011. *Histology: A text and atlas: with correlated cell and molecular biology,* Philadelphia, PA, Wolters Kluwer/ Lippincott Williams & Wilkins.

ROSS, R., SHAW, K. D., MARTEL, Y., DE GUISE, J. & AVRUCH, L. 1993. Adipose tissue distribution measured by magnetic resonance imaging in obese women. *Am J Clin Nutr,* 57**,** 470-5.

ROTHENBERG, E. 1994. Validation of the food frequency questionnaire with the 4-day record method and analysis of 24-h urinary nitrogen. *Eur J Clin Nutr,* 48**,** 725-35.

ROWE, J. W., YOUNG, J. B., MINAKER, K. L., STEVENS, A. L., PALLOTTA, J. & LANDSBERG, L. 1981. Effect of insulin and glucose infusions on sympathetic nervous system activity in normal man. *Diabetes,* 30**,** 219-25.

SAIVASANTHI, V., GOWTHAMIGOUD., SWATHI, K., AAKRUTHI., SOWMYA, R., GUPTA, A. & ROA, A. S. 2011. Evaluation of Caralluma fimbriata for analgesic, antiinflammatory and anxiolytic activities. *Int J Pharm,* 1.

SALAMONE, F., LI VOLTI, G., TITTA, L., PUZZO, L., BARBAGALLO, I., LA DELIA, F., ZELBER-SAGI, S., MALAGUARNERA, M., PELICCI, P. G., GIORGIO, M. & GALVANO, F. 2012. Moro orange juice prevents fatty liver in mice. *World J Gastroenterol,* 18**,** 3862-8.

SALVAMANI, S., GUNASEKARAN, B., SHAHARUDDIN, N. A., AHMAD, S. A. & SHUKOR, M. Y. 2014. Antiartherosclerotic effects of plant flavonoids. *Biomed Res Int,* 2014**,** 480258.

SANTOS, A. C., EBRAHIM, S. & BARROS, H. 2007. Alcohol intake, smoking, sleeping hours, physical activity and the metabolic syndrome. *Prev Med* 44**,** 328-34.

SANTOS, A. P., ROGERO, M. M. & BASTOS, D. H. 2010. Edible plants, their secondary metabolites and antiobesogenic potential. *Recent Pat Food Nutr Agric,* 2**,** 195-212.

SAYANTAN, R. & ABHISHEK, S. 2012. Antidiabetic activity of Caralluma edulis bark and leaf extract against streptozotocin induced diabetic rats. *J Pharm Healthc Manag,* 3**,** 76-81.

SEIDELL, J. C., PERUSSE, L., DESPRES, J. P. & BOUCHARD, C. 2001. Waist and hip circumferences have independent and opposite effects on cardiovascular disease risk factors: the Quebec Family Study. *Am J Clin Nutr,* 74**,** 315-21.

SHAJEELA, P., KALPANADEVI, V. & MOHAN, V. 2013. Potential antidiabetic, hypolipidaemic and antioxidant effects of Xanthosoma sagittifolium extract in alloxan induced diabetic rats. *Int J Pharm Pharm Sci,* 5**,** 27-31.

SHARMA, A. M. & CHETTY, V. T. 2005. Obesity, hypertension and insulin resistance. *Acta Diabetol,* 42 Suppl 1**,** S3-8.

SHIBASAKI, T., ODA, T., IMAKI, T., LING, N. & DEMURA, H. 1993. Injection of anti-neuropeptide Y gamma-globulin into the hypothalamic paraventricular nucleus decreases food intake in rats. *Brain Res,* 601**,** 313-6.

SHIH, P. H., YEH, C. T. & YEN, G. C. 2007. Anthocyanins induce the activation of phase II enzymes through the antioxidant response element pathway against oxidative stress-induced apoptosis. *J Agric Food Chem,* 55**,** 9427-35.

SHINDO, M., KASAI, T., ABE, A. & KONDO, Y. 2007. Effects of dietary administration of plant-derived anthocyanin-rich colors to spontaneously hypertensive rats. *J Nutr Sci Vitaminol (Tokyo),* 53**,** 90-3.

SHIVANI S & SUNIL, S. 2013. Antidiabetic effect of Helianthus Annuus L., seeds ethanolic extract in streptozotocin-nicotinamide induced type 2 diabetes mellitus. *Int J Pharm Pharm Sci,* 5**,** 382-387.

SIEGRIST-KAISER, C. A., PAULI, V., JUGE-AUBRY, C. E., BOSS, O., PERNIN, A., CHIN, W. W., CUSIN, I., ROHNER-JEANRENAUD, F., BURGER, A. G., ZAPF, J. & MEIER, C. A. 1997. Direct effects of leptin on brown and white adipose tissue. *J Clin Invest,* 100**,** 2858-64.

SKILTON, M. R., MOULIN, P., SERUSCLAT, A., NONY, P. & BONNET, F. 2007. A comparison of the NCEP-ATPIII, IDF and AHA/NHLBI metabolic syndrome definitions with relation to early carotid atherosclerosis in subjects with

hypercholesterolemia or at risk of CVD: evidence for sex-specific differences. *Atherosclerosis,* 190**,** 416-22.

SNIDERMAN, A. D., BHOPAL, R., PRABHAKARAN, D., SARRAFZADEGAN, N. & TCHERNOF, A. 2007. Why might South Asians be so susceptible to central obesity and its atherogenic consequences? The adipose tissue overflow hypothesis. *Int J Epidemiol,* 36**,** 220-5.

SNIJDER, M. B., ZIMMET, P. Z., VISSER, M., DEKKER, J. M., SEIDELL, J. C. & SHAW, J. E. 2004a. Independent and opposite associations of waist and hip circumferences with diabetes, hypertension and dyslipidemia: the AusDiab Study. *International journal of obesity and related metabolic disorders,* 28**,** 402-9.

SNIJDER, M. B., ZIMMET, P. Z., VISSER, M., DEKKER, J. M., SEIDELL, J. C. & SHAW, J. E. 2004b. Independent association of hip circumference with metabolic profile in different ethnic groups. *Obesity Research* 12**,** 1370-4.

SOMNATH, S., PATIL, S. D. & SANJAY, S. 2012. Hypolipidemia activity of Caralluma adscendens on Triton and methimazole induced hyperlipidemia rats. *Pharm tech medica,* 1.

SONI, M. G., BURDOCK, G. A., PREUSS, H. G., STOHS, S. J., OHIA, S. E. & BAGCHI, D. 2004. Safety assessment of (-)-hydroxycitric acid and Super CitriMax, a novel calcium/potassium salt. *Food Chem Toxicol,* 42**,** 1513-29.

SPITZER, R. L., YANOVSKI, S., WADDEN, T., WING, R., MARCUS, M. D., STUNKARD, A., DEVLIN, M., MITCHELL, J., HASIN, D. & HORNE, R. L. 1993. Binge eating disorder: its further validation in a multisite study. *Int J Eat Disord,* 13**,** 137-53.

STEWART, K. J. 2002. Exercise training and the cardiovascular consequences of type 2 diabetes and hypertension: plausible mechanisms for improving cardiovascular health. *J Am Med Assoc*, 288**,** 1622-31.

STRITTMATTER, S. M. & SNYDER, S. H. 1986. Characterization of angiotensin converting enzyme by [3H]captopril binding. *Mol Pharmacol*, 29**,** 142-8.

SU, X. Q., ANTONAS, K. N. & LI, D. 2004. Comparison of n-3 polyunsaturated fatty acid contents of wild and cultured Australian abalone. *Int J Food Sci Nutr*, 55**,** 149-54.

SUDHAKARA, G., MALLAIAH, P., SREENIVASULU, N., SASI BHUSANA RAO, B., RAJENDRAN, R. & SARALAKUMARI, D. 2014. Beneficial effects of hydro-alcoholic extract of Caralluma fimbriata against high-fat diet-induced insulin resistance and oxidative stress in Wistar male rats. *J Physiol Biochem*.

SULLIVAN, A. C., TRISCARI, J., HAMILTON, J. G. & MILLER, O. N. 1974a. Effect of (-)-hydroxycitrate upon the accumulation of lipid in the rat. II. Appetite. *Lipids*, 9**,** 129-34.

SULLIVAN, A. C., TRISCARI, J., HAMILTON, J. G., MILLER, O. N. & WHEATLEY, V. R. 1974b. Effect of (-)-hydroxycitrate upon the accumulation of lipid in the rat. I. Lipogenesis. *Lipids*, 9**,** 121-8.

SUMITHRAN, P., PRENDERGAST, L. A., DELBRIDGE, E., PURCELL, K., SHULKES, A., KRIKETOS, A. & PROIETTO, J. 2011. Long-term persistence of hormonal adaptations to weight loss. *N Engl J Med*, 365**,** 1597-604.

TARGHER, G., BERTOLINI, L., POLI, F., RODELLA, S., SCALA, L., TESSARI, R., ZENARI, L. & FALEZZA, G. 2005. Nonalcoholic fatty liver disease and risk of future cardiovascular events among type 2 diabetic patients. *Diabetes*, 54**,** 3541-6.

TATIYA, A., KULKARNI, A., SURANA, S. & BARI, N., . 2010. Antioxidant and Hypolipidemic Effect of Caralluma adscendens Roxb. in Alloxanized Diabetic Rats. *Int J Pharm,* 6**,** 400-406.

THE NATIONAL CHOLESTEROL EDUCATION PROGRAM 2001. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *J Am Med Assoc,* 285**,** 2486-97.

THOMAS, B. 2001. *Manual of Dietetic Practice,* Melbourne, Blackwell Publishing.

THONGPRADICHOTE, S., MATSUMOTO, K., TOHDA, M., TAKAYAMA, H., AIMI, N., SAKAI, S. & WATANABE, H. 1998. Identification of opioid receptor subtypes in antinociceptive actions of supraspinally-administered mitragynine in mice. *Life Sci* 62**,** 1371-8.

TITTA, L., TRINEI, M., STENDARDO, M., BERNIAKOVICH, I., PETRONI, K., TONELLI, C., RISO, P., PORRINI, M., MINUCCI, S., PELICCI, P. G., RAPISARDA, P., REFORGIATO RECUPERO, G. & GIORGIO, M. 2010. Blood orange juice inhibits fat accumulation in mice. *Int J Obes (Lond),* 34**,** 578-88.

TOSETTI, C., CORINALDESI, R., STANGHELLINI, V., PASQUALI, R., CORBELLI, C., ZOCCOLI, G., DI FEBO, G., MONETTI, N. & BARBARA, L. 1996. Gastric emptying of solids in morbid obesity. *Int J Obes Relat Metab Disord,* 20**,** 200-5.

TREVISAN, M., LIU, J., BAHSAS, F. B. & MENOTTI, A. 1998. Syndrome X and mortality: a population-based study. Risk Factor and Life Expectancy Research Group. *Am J Epidemiol,* 148**,** 958-66.

TRINDER, P. 1969. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Ann Clin Biochem,* 6**,** 24-27.

TRISCARI, J. & SULLIVAN, A. C. 1977. Comparative effects of (--)-hydroxycitrate and (+)-allo-hydroxycitrate on acetyl CoA carboxylase and fatty acid and cholesterol synthesis in vivo. *Lipids,* 12**,** 357-63.

TSUDA, T. 2008. Regulation of adipocyte function by anthocyanins; possibility of preventing the metabolic syndrome. *J Agric Food Chem,* 56**,** 642-6.

TSUDA, T., HORIO, F., UCHIDA, K., AOKI, H. & OSAWA, T. 2003. Dietary cyanidin 3-O-beta-D-glucoside-rich purple corn color prevents obesity and ameliorates hyperglycemia in mice. *J Nutr,* 133**,** 2125-30.

TSUDA, T., UENO, Y., AOKI, H., KODA, T., HORIO, F., TAKAHASHI, N., KAWADA, T. & OSAWA, T. 2004. Anthocyanin enhances adipocytokine secretion and adipocyte-specific gene expression in isolated rat adipocytes. *Biochem Biophys Res Commun,* 316**,** 149-57.

TSUDA, T., UENO, Y., KOJO, H., YOSHIKAWA, T. & OSAWA, T. 2005. Gene expression profile of isolated rat adipocytes treated with anthocyanins. *Biochim Biophys Acta,* 1733**,** 137-47.

TSUDA, T., UENO, Y., YOSHIKAWA, T., KOJO, H. & OSAWA, T. 2006. Microarray profiling of gene expression in human adipocytes in response to anthocyanins. *Biochem Pharmacol,* 71**,** 1184-97.

URANO, T., KOJIMA, Y., TAKAHASHI, M., SERIZAWA, K., SAKAKIBARA, K., TAKADA, Y. & TAKADA, A. 1993. Impaired fibrinolysis in hypertension and obesity due to high plasminogen activator inhibitor-1 level in plasma. *Jpn J Physiol,* 43**,** 221-8.

VAGUE, J. 1947. La differenciation sexuelle, facteur determinant des formes de l'obesité. *Presse Medl,* 53**,** 339-340.

VAN HARMELEN, V., DICKER, A., RYDEN, M., HAUNER, H., LONNQVIST, F., NASLUND, E. & ARNER, P. 2002. Increased lipolysis and decreased leptin production by human omental as compared with subcutaneous preadipocytes. *Diabetes,* 51**,** 2029-36.

VAN HEERDEN, F. R., HORAK, R.M., MAHARAJ, V.J., VLEGGAAR, R., SENABE, J.V., GUNNING, & P.J. 2007. An appetite suppressant from Hoodia species. *Phytochemistry,* 68.

VENKATESH, R., RAJENDRAN, R. 2007. Role of Caralluma fimbriata in weight management. *In:* BAGCHI, D., PREUSS, H. *Obesity: Epidemiology, Pathophysiology, and Prevention.* CRC Press Boca Ration.

VERMA, S., LI, S. H., WANG, C. H., FEDAK, P. W., LI, R. K., WEISEL, R. D. & MICKLE, D. A. 2003. Resistin promotes endothelial cell activation: further evidence of adipokine-endothelial interaction. *Circulation,* 108**,** 736-40.

VERWEIJ, L. M., TERWEE, C. B., PROPER, K. I., HULSHOF, C. T. & VAN MECHELEN, W. 2013. Measurement error of waist circumference: gaps in knowledge. *Public Health Nutr,* 16**,** 281-8.

VIERHAPPER, H. 1985. Effect of exogenous insulin on blood pressure regulation in healthy and diabetic subjects. *Hypertension,* 7**,** II49-53.

VINEGAR, R., SCHREIBER, W. & HUGO, R. 1969. Biphasic development of carrageenin edema in rats. *J Pharmacol Exp Ther,* 166**,** 96-103.

VINOD, K., KHOMEDNDRA, K. & KAMARUZ, Z. 2013. Antihyperglycemic activity of Swertia chirayita and Andrographis paniculata plant extracts in streptozotocin induced diabetic rats. *Int J Pharm Pharm Sci,* 5**,** 305-311.

VIRDIS, A., IGLARZ, M., NEVES, M. F., AMIRI, F., TOUYZ, R. M., ROZEN, R. & SCHIFFRIN, E. L. 2003. Effect of hyperhomocystinemia and hypertension on

endothelial function in methylenetetrahydrofolate reductase-deficient mice. *Arterioscler Thromb Vasc Biol,* 23**,** 1352-7.

VOLANAKIS, J. E. 2001. Human C-reactive protein: expression, structure, and function. *Mol Immunol,* 38**,** 189-97.

VOZAROVA, B., STEFAN, N., LINDSAY, R. S., SAREMI, A., PRATLEY, R. E., BOGARDUS, C. & TATARANNI, P. A. 2002. High alanine aminotransferase is associated with decreased hepatic insulin sensitivity and predicts the development of type 2 diabetes. *Diabetes,* 51**,** 1889-95.

VOZAROVA, B., WEYER, C., HANSON, K., TATARANNI, P. A., BOGARDUS, C. & PRATLEY, R. E. 2001. Circulating interleukin-6 in relation to adiposity, insulin action, and insulin secretion. *Obes Res,* 9**,** 414-7.

WADOOD, A., WADOOD, N. & SHAH, S. A. 1989. Effects of Acacia arabica and Caralluma edulis on blood glucose levels of normal and alloxan diabetic rabbits. *J Pak Med Assoc,* 39**,** 208-12.

WAGENKNECHT, L. E., LANGEFELD, C. D., SCHERZINGER, A. L., NORRIS, J. M., HAFFNER, S. M., SAAD, M. F. & BERGMAN, R. N. 2003. Insulin sensitivity, insulin secretion, and abdominal fat: the Insulin Resistance Atherosclerosis Study (IRAS) Family Study. *Diabetes,* 52**,** 2490-6.

WALTER, M. J., SCHERRER, J. F., FLOOD, J. F. & MORLEY, J. E. 1994. Effects of localized injections of neuropeptide Y antibody on motor activity and other behaviors. *Peptides,* 15**,** 607-13.

WANG, M. Y., LEE, Y. & UNGER, R. H. 1999. Novel form of lipolysis induced by leptin. *J Biol Chem,* 274**,** 17541-4.

WEINBRENNER, T., SCHRODER, H., ESCURRIOL, V., FITO, M., ELOSUA, R., VILA, J., MARRUGAT, J. & COVAS, M. I. 2006. Circulating oxidized LDL is associated

with increased waist circumference independent of body mass index in men and women. *Am J Clin Nutr,* 83**,** 30-5; quiz 181-2.

WEYER, C., FUNAHASHI, T., TANAKA, S., HOTTA, K., MATSUZAWA, Y., PRATLEY, R. E. & TATARANNI, P. A. 2001. Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. *J Clin Endocrinol Metab,* 86**,** 1930-5.

WHELTON, P. K. 1996. Primary prevention of hypertension: rationale, approaches, realities and perspectives. *Journal of Human Hypertension,* 10 Suppl 1**,** S47-50.

WHITNEY E & ROLFES S 2008. *Understanding Nutrition,* Australia, Thomson Wadsworth.

WILSON, A. M., SWAN, J. D., DING, H., ZHANG, Y., WHITBOURN, R. J., GURRY, J., YII, M., WILSON, A. C., HILL, M., TRIGGLE, C., BEST, J. D. & JENKINS, A. J. 2007. Widespread vascular production of C-reactive protein (CRP) and a relationship between serum CRP, plaque CRP and intimal hypertrophy. *Atherosclerosis,* 191**,** 175-81.

WITZTUM, J. L. 1994. The oxidation hypothesis of atherosclerosis. *Lancet,* 344**,** 793-5.

WOLPERT, H. A., STEEN, S. N., ISTFAN, N. W. & SIMONSON, D. C. 1993. Insulin modulates circulating endothelin-1 levels in humans. *Metabolism,* 42**,** 1027-30.

WORLD HEALTH ORGANISATION. 2015. *Global Health Observatory (GHO): Obesity* [Online]. 12/01/15].

WORLD HEALTH ORGANIZATION 1999. World Health Organization, Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications; Part 1: Diagnosis and Classification of Diabetes Mellitus. *In:* DEPARTMENT OF NONCOMMUNICABLE DISEASE SURVEILLANCE (ed.). Geneva.

WORLD HEALTH ORGANIZATION 2000a. The Asia-Pacific Perpective. Redefining obesity and its treatment *World Health Organ Tech Rep Ser*.

WORLD HEALTH ORGANIZATION 2000b. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. *World Health Organization technical report series,* 894**,** i-xii, 1-253.

WORLD HEALTH ORGANIZATION 2000c. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. *World Health Organ Tech Rep Ser,* 894**,** i-xii, 1-253.

WU, X., MOTOSHIMA, H., MAHADEV, K., STALKER, T. J., SCALIA, R. & GOLDSTEIN, B. J. 2003. Involvement of AMP-activated protein kinase in glucose uptake stimulated by the globular domain of adiponectin in primary rat adipocytes. *Diabetes,* 52**,** 1355-63.

XYRIS SOFTWARE PTY LTD 2007. Food Works Professional. Highgate Hill, QLD, Australia.

YAMAUCHI, T., KAMON, J., WAKI, H., TERAUCHI, Y., KUBOTA, N., HARA, K., MORI, Y., IDE, T., MURAKAMI, K., TSUBOYAMA-KASAOKA, N., EZAKI, O., AKANUMA, Y., GAVRILOVA, O., VINSON, C., REITMAN, M. L., KAGECHIKA, H., SHUDO, K., YODA, M., NAKANO, Y., TOBE, K., NAGAI, R., KIMURA, S., TOMITA, M., FROGUEL, P. & KADOWAKI, T. 2001. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat Med,* 7**,** 941-6.

YANG, W. S., LEE, W. J., FUNAHASHI, T., TANAKA, S., MATSUZAWA, Y., CHAO, C. L., CHEN, C. L., TAI, T. Y. & CHUANG, L. M. 2001. Weight reduction increases plasma levels of an adipose-derived anti-inflammatory protein, adiponectin. *J Clin Endocrinol Metab,* 86**,** 3815-9.

YATAGAI, T., NAGASAKA, S., TANIGUCHI, A., FUKUSHIMA, M., NAKAMURA, T., KUROE, A., NAKAI, Y. & ISHIBASHI, S. 2003. Hypoadiponectinemia is associated

with visceral fat accumulation and insulin resistance in Japanese men with type 2 diabetes mellitus. *Metabolism,* 52**,** 1274-8.

YEBOAH, J., CROUSE, J. R., HSU, F. C., BURKE, G. L. & HERRINGTON, D. M. 2007. Brachial flow-mediated dilation predicts incident cardiovascular events in older adults: the Cardiovascular Health Study. *Circulation,* 115**,** 2390-7.

YOKOTA, T., ORITANI, K., TAKAHASHI, I., ISHIKAWA, J., MATSUYAMA, A., OUCHI, N., KIHARA, S., FUNAHASHI, T., TENNER, A. J., TOMIYAMA, Y. & MATSUZAWA, Y. 2000. Adiponectin, a new member of the family of soluble defense collagens, negatively regulates the growth of myelomonocytic progenitors and the functions of macrophages. *Blood,* 96**,** 1723-32.

YU, C., CHEN, Y., CLINE, G. W., ZHANG, D., ZONG, H., WANG, Y., BERGERON, R., KIM, J. K., CUSHMAN, S. W., COONEY, G. J., ATCHESON, B., WHITE, M. F., KRAEGEN, E. W. & SHULMAN, G. I. 2002. Mechanism by which fatty acids inhibit insulin activation of insulin receptor substrate-1 (IRS-1)-associated phosphatidylinositol 3-kinase activity in muscle. *J Biol Chem,* 277**,** 50230-6.

YULIANA, N. D., JAHANGIR, M., KORTHOUT, H., CHOI, Y. H., KIM, H. K. & VERPOORTE, R. 2011. Comprehensive review on herbal medicine for energy intake suppression. *Obesity Rev* 12**,** 499-514.

YUN, J. E., KIMM, H., JO, J. & JEE, S. H. 2010. Serum leptin is associated with metabolic syndrome in obese and nonobese Korean populations. *Metabolism,* 59**,** 424-9.

ZAKARIA, M. N., ISLAM, M. W., RADHAKRISHNAN, R., CHEN, H. B., KAMIL, M., AL-GIFRI, A. N., CHAN, K. & AL-ATTAS, A. 2001. Anti-nociceptive and anti-inflammatory properties of Caralluma arabica. *J Ethnopharmacol,* 76**,** 155-8.

ZHANG, Y., PROENCA, R., MAFFEI, M., BARONE, M., LEOPOLD, L. & FRIEDMAN, J. M. 1994. Positional cloning of the mouse obese gene and its human homologue. *Nature*, 372**,** 425-32.

Appendix 1 - A Review on Botanical Species and Chemical Compounds with Appetite Suppressing Properties for Body Weight Control

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ORIGINAL PAPER

A Review on Botanical Species and Chemical Compounds with Appetite Suppressing Properties for Body Weight Control

Katie J. Astell · Michael L. Mathai · Xiao Q. Su

Abstract As obesity has reached epidemic proportions, the management of this global disease is of clinical importance. The availability and popularity of natural dietary supplements for the treatment of obesity has risen dramatically in recent years. The purpose of this paper was to review the effect of commonly available over the counter plant-derived supplements used to suppress appetite for obesity control and management. The data were obtained from the electronic databases PubMed, SpringerLink, Google Scholar, ScienceDirect, and MEDLINE with full text (via EBSCOHost) and the databases were accessed during late 2012 - early January 2013. The botanical species discussed in this review include *Camellia sinensis, Caralluma fimbriata, Citrus aurantium, Coleus forskohlii, Garcinia cambogia and Phaseolus vulgaris.* This review found that many botanical species including crude extracts and isolated compounds from plants have been shown to provide potentially promising therapeutic effects including appetite control and weight loss. However, many of these crude extracts and compounds need to be further investigated to define the magnitude of the effects, optimal dosage, mechanisms of action, long term safety, and potential side effects.

Keywords Botanical species · Appetite · Food intake · Obesity · Natural supplements

Abbreviations

ATP	Adenosine triphosphate
GRAS	Generally recognized as safe
SNS	Sympathetic nervous system
WHR	Waist to hip ratio

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Introduction

Overweight and obesity have reached epidemic proportions and are considered a serious health problem, increasing the global burden of chronic lifestyle related diseases, such as type 2 diabetes and cardiovascular disease. Therefore the treatment of overweight and obesity is of clinical importance. At present, the global strategy may involve a combination of therapies including reducing energy intake, increasing energy expenditure, behavioural modification, pharmacotherapy and even surgery to counteract obesity development [1].

The use of medicinal plant extracts for weight loss is a rapidly growing therapeutic area, which has been embraced by the general public. These products contain many dietary phytochemical constituents with great potential for chronic disease prevention and treatment [2]. Therefore, the possibility of using these natural supplements for the long term treatment of obesity has aroused considerable interest and is now under exploration. Although, the popularity of natural supplements has risen dramatically in recent years, the clinical efficacy of these supplements still remains uncertain in most cases. In addition, there is over-hype of the utility of many weight loss aids without providing supporting evidence. Therefore, this paper will review the most recent evidence on plant-derived supplements that are currently available over the counter that potentially have appetite suppressing properties. Moreover, data collected from studies on ethnobotany, efficacy, adverse events, chemical constituents, pharmacology, mechanism of action and changes in food intake and body composition are described. Natural ingredients including crude extracts and isolated compounds

in plants are an alternative strategy for obesity treatment. Plant based supplements marketed as natural appetite suppressants and weight loss aids include *C. sinensis, C. fimbriata, C. aurantium, C. forskohlii, G. cambogia and P. vulgaris.*

Botanical Species and Chemical Compounds Possessing Appetite Suppressing Properties

Camellia sinensis

Green tea is brewed from unfermented dried leaves of the *C. sinensis* plant. The leaves are pan-fried or steamed during processing to inhibit polyphenol oxidase activity. Chemically, green tea is categorized by the occurrence of large amounts of polyphenols known as catechins. Polyphenols including flavonols, flavones, and flavan-3-ols are a rich source of green tea representing approximately 30 % of the dry weight of the fresh leaf [3]. 60–80 % of these polyphenols are flavan-3-ols which are referred as catechins. A typical cup of brewed green tea contains 240–320 mg of catechins with epigallocatechin-3-gallate accounting for 30–50 % of them. The most abundant catechin of green tea is epigallocatechin-3-gallate, accounting for 50–80 % of the total catechin concentration. This catechin is also known to be the most bioactive component of green tea [4]. Catechin, epicatechin, epigallocatechin and epicatechin-3-gallate are minor isomers of this compound. The remaining ingredients in green tea include quercetin, theorubigins, theaflavins, theanine, caffeine, and other phenolics including chlorogenic acid and gallic acid.

The mechanism of action of green tea catechins remains an active area of exploration. It is hypothesized that green tea catechins influence the SNS activity, elevating energy expenditure and thereby stimulating fat oxidation (Fig. 1) [3]. Other possible mechanisms include appetite inhibition, reduction of nutrient absorption and up-regulation of enzymes involved in hepatic fat oxidation (Fig. 1) [3].

Studies have shown that substances such as beta-adrenergic agonists known to enhance hepatic fatty acid oxidation are capable of reducing food intake in rats [5]. It is suggested that energy production namely ATP in the liver stimulates signals to the appetite-regulating centres of the brain via vagal sensory neurons [6]. Therefore, appetite is increased as a result of low hepatic fatty acid oxidation and decreased ATP levels. A reduction in food intake has been observed in human participants following consumption of medium-chain fatty acids and 1, 3-diacylglyceride oil, which are compounds known to elevate hepatic fatty acid oxidation [7]. Evidence suggests that green tea catechins may

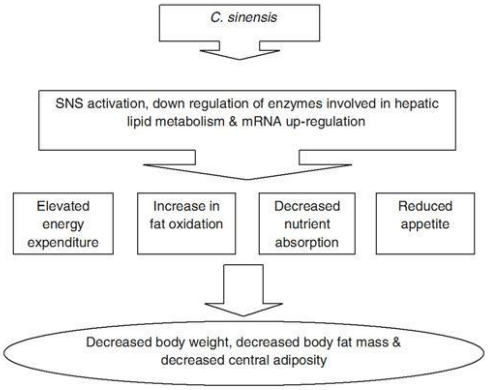

Fig. 1 Proposed antiobesity mechanisms of *C. sinensis*

potentially increase hepatic fat oxidation and ATP production and thus it was hypothesized that appetite could be altered following ingestion of green tea catechins.

There are inconsistent findings with regards to the effect of epigallocatechin-3-gallate or green tea catechins on food intake in animal studies [4, 8, 9]. Following oral administration of green tea catechins or pure epigallocatechin-3-gallate there was no change in food intake [10–15]. However, Kao et al. [9] demonstrated that administration of epigallocatechin-3-gallate via intraperitoneal injection significantly reduced food intake by 50–60 % compared to the control rats. In addition, a study conducted by Murase et al. [8] also found that 0.5 % tea catechins-fed mice significantly reduced energy intake by 5.6 %.

Positive findings for *C. sinensis* on appetite sensations and energy intake in human clinical trials are lacking. Several studies have failed to confirm a treatment effect in the experimental group on appetite or energy intake compared to the placebo group [16–21]. Common methods used to measure subjective appetite profile and dietary intake in these studies were via the visual analogue scales method [16, 20, 21], food diary records [16, 17, 21] and the three-factor eating questionnaire [16]. Possible methodological flaws in these studies may have influenced the outcome of the results. For example, in some studies caffeine consumption was not controlled during the study period [16, 17]. The most common minor adverse events reported following supplementation of catechins include joint pain, sinusitis and rhinitis [18]. Overall, the evidence on *C. sinesis* as an appetite suppressant is not compelling. However, it can be concluded from the majority of these studies that *C. sinensis* is capable of reducing body weight through

other mechanisms such as increasing energy expenditure [17, 18, 20, 21].

Caralluma fimbriata

C. fimbriata is an edible succulent plant, in the Apocynaceae family, native to India. *C. fimbriata* has been used for its appetite suppressing effects and as a portable food for hunting [22]. The edible cactus flourishes in the states of Andhra Pradesh, Karnataka, and Tamil Nadu of India as a roadside shrub and is planted as a boundary marker in gardens. Indian tribal people have included the cactus in their diet for many centuries. Among Indian tribal populations *C. fimbriata* is known as a famine food and thirst quencher when its green follicles are boiled and salted [22].

The phytochemistry of the genus *Caralluma* is characterized by many pregnane glycosides. Other chemical constituents include flavones glycosides, megastigmane glycosides, saponins and several flavonoids. The appetite suppressing properties of *C. fimbriata* could be attributed to the pregnane glycosides, which are present in the plant species belonging to the Apocynaceae family [23]. Eleven pregnane glycosides have been isolated from the plant extract with four (compounds 10–13) containing a novel genin.

It is uncertain how pregnane glycosides may suppress appetite; it is thought that the pregnane glycosides amplify the signalling of energy sensing function in the hypothalamus [22]. Another hypothesis is that *C. fimbriata* may down-regulate ghrelin synthesis in the stomach and neuropeptide-Y in the hypothalamus, resulting in appetite suppression [24]. *Hoodia gordonii* has been reported to have a similar appetite suppressing action as *C. fimbriata*, in which a steroidal glycoside was isolated, which displayed anorectic activity in animals [22].

A rat study investigating the anti-obesogenic and anti-atherosclerotic properties of *C. fimbriata* extract showed that following oral administration, there was a significant reduction in food intake and the extract inhibited increases in body weight, liver weight and fat pad mass [25]. There is limited research into the effect of *C. fimbriata* on appetite in humans. A human trial conducted on the appetite suppressing effects of *C. fimbriata* in Indian adults found that the extract (1 g/day) appears to suppress appetite and reduce waist circumference in overweight individuals (n=50) with a BMI greater than 25 kg/m^2 over a two month period compared to the placebo group [22]. Kuriyan et al. [22] also found that hunger levels of participants reduced by 20 % following the administration period, which may account for an 8 % decline in energy intake of the experimental group. Appetite sensations including 'hunger', 'thoughts of food', 'urge to eat' and 'fullness of stomach' were assessed by the visual analogue scales method and dietary intake was assessed via a modified food frequency questionnaire. The food frequency questionnaire indicated that the appetite suppressing effect caused a decrease in energy and fat intake and also a decline in the consumption of less desirable food [22]. The reduction in less desirable foods such as sugar and sweets indicates that the reward circuit interruption is involved in the appetite suppressant activity. Minor adverse events experienced in this study include abdominal distention, flatulence, gastritis and constipation [22].

Our recent human trial investigated the effect of *C. fimbriata* extract in combination with controlled dietary intake and physical activity on the risk factors of metabolic syndrome in overweight and obese Australian subjects [26]. The primary outcome was the marked decline in waist circumference and WHR after 12 weeks intervention in the experimental group. However, hunger levels did not change in the experimental group, even though there was a significant reduction in the palatability of the test meal [26]. Minor adverse events experienced in the experimental group included a dermatological rash and constipation. Therefore, *C. fimbriata* extract has received the Generally Recognized As Safe (GRAS) status for use as a nutraceutical for obesity treatment [27]. These positive clinical trials provide sufficient evidence that *C. fimbriata* is capable of curbing central obesity. However, further studies are required to determine the efficacy of *C. fimbriata* as an appetite suppressant.

Citrus aurantium

C. aurantium has several common names such as bitter orange, sour orange, Seville orange, zhishi, chongcao, and neroli. The citrus plant is native to tropical Asia and is also found in other tropical and subtropical countries especially Spain. *C. aurantium* is quite sour and therefore not popular for eating, however, the ripe fruit is eaten in Iran and the fresh fruit is eaten in Mexico with added salt and chili. The fruit is sometimes used for culinary purposes but most often the plant is used for herbal medicine to suppress appetite. With the ban of ephedra-containing dietary supplements, the bitter orange has been labelled as a replacement without the adverse side effects of ephedra. However, there is no evidence to suggest that it is a safer alternative to ephedra.

The bitter orange is known for the volatile oil in the peel. This volatile oil is part of the residue that is present after peeling citrus fruit. This oil gives bitter orange its strong flavor and odor and also accounts for several of its therapeutic uses. The peel also contains other phytochemicals besides the volatile oil. The main bioactive constituents in the plant are flavonoids, particularly hesperidin, naringin and alkaloids namely synephrine [28]. Like ephedra, *Citrus aurantium* contains alkaloids that are adrenergic agonists

and are typically incorporated in supplements to help aid weight loss. The alkaloids present in *C. aurantium* are thought to mainly be α-adrenergic agonists, however they may also contain β-adrenergic agonist properties. *C. aurantium* contains many phytochemicals including para-octopamine and synephrine alkaloids. These phytochemicals are adrenergic agonists and are considered to be active ingredients. Para-synephrine is an α-adrenergic agonist and has some β-adrenergic properties. Para-synephrine is also referred as oxedrine and is typically used in eye drops. Oxedrine is believed to be the chief ingredient in *C. aurantium* that stimulates weight loss. Meta-synephrine also referred as phenylephrine is an α-adrenergic agonist but also has some β-adrenergic agonist properties and is widely used as a decongestant [29].

It has been suggested that the synephrine alkaloids may potentially decrease food intake and increase energy expenditure due to the fact that synephrine alkaloids are a sympathomimentric agent containing both α- and β-adrenergic receptor agonists [30]. It is also evident that adrenergic agonists such as synephrine alkaloids reduce gastric motility. Similarly, cholecystokinin and other gut peptides reduce food intake through decreased gastric motility [31], thus it has been postulated that synephrine alkaloids may also reduce food intake through the reduction in gut motility.

It is well documented in animal trials that synephrine alkaloids significantly reduced food intake in rodents [32]. However, there is little evidence to support the use of *C. aurantium* for appetite control and weight loss in humans, despite its popular presence in over the counter weight loss products. Two pilot studies conducted by Greenway et al. [33] failed to identify a significant difference between the treatment and placebo groups for food intake, appetite ratings and body composition [33]. Dietary intake and appetite ratings were only measured in the first pilot study via the visual analogue scales method and a questionnaire about factors that may affect taste such as allergies or a cold was completed. Adverse events reported in these pilot studies include: cardiovascular hypertension, dermatological harm, diarrhea, nausea/vomiting, menstrual cramps, headaches, migraines, insomnia, anxiety, flu-like symptoms, oral complaints, and upper respiratory problems. The current research for *C. aurantium* on appetite and energy intake does not seem to be a promising strategy for suppressing appetite for body weight reduction.

Coleus forskohlii

C. forskohlii is a native Indian Coleus plant that grows wild in arid and semi-arid regions of India and Thailand. Forskolin is a labdane diterpene isolated from the roots of the Indian based *C. forskohlii*. *C. forskohlii* Briq (Lamiaceae) is considered the most common species that contains forskolin. *C. forskohlii* Briq belongs to the Labiatae family (mint family). As Forskolin is a diterpene it acts directly on adenylatecyclase. This enzyme activates cAMP which then stimulates fat breakdown in human and animal fat cells [34]. cAMP increases utilization of body fat, increases basal metabolic rate, and regulates the body's thermogenic response to food. Fatty acids may be released from adipose tissue depots through cAMP which causes an increase in thermogenesis, loss of body fat and an increase in lean tissue. An increase in cAMP accumulation is associated with high concentrations of Forskolin, which thus enhances lipolysis. These effects may lead to fat loss without muscle mass loss with supplementation of Forskolin [35].

Animal studies have shown that administration with *C. forskolhii* significantly reduces food intake [36]. *C. forskolhii* has also been shown to affect appetite in humans. A study conducted by Henderson et al. [37] evaluated the effects of *C. forskolhii* supplementation on body composition and haematological profiles in mildly overweight women. According to the food diary records, there was no significant difference between the *C. forskohlii* and placebo groups in terms of mean daily energy intake and fat, carbohydrate and protein intakes. However, the appetite assessment via the visual analogue scales method revealed a significant decrease in feelings of fullness in the *C. forskohlii* group from week 0 to week 12. In addition, there was a significant reduction in the satisfaction of food consumed in both groups, suggesting that there was less enjoyment in eating and therefore less food was consumed [37]. No adverse events were reported during the intervention period. Another study conducted by Godard et al. [35] investigating the effects of *C. forskolhii* supplementation on body composition and hormonal adaptations also did not show a significant difference in mean daily calorie intake between the *C. forskohlii* and placebo groups which was obtained by a dietary recall. Overall, the evidence on *C. forskohlii* as an appetite suppressant and weight loss agent is inconsistent. Further research is required to elucidate the pharmacotherapy and bioactivity of the extract.

Garcinia cambogia

Hydroxycitric acid is a popular dietary supplement from the dried fruit rind of *G. cambogia*. This tree is native to Southeast Asia and belongs to the Guttiferae family. *G. cambogia* is also referred to as Malabar tamarind, which is used extensively for culinary purposes in southern India. Studies have observed that sprinkling the dried fruit rind on curries makes the food more satisfying, stimulating the feeling of fullness. In terms of structure, hydroxycitric acid

is chemically similar to citric acid. The fruit of *G. cambogia* contains about 10–30 % acid which is calculated as citric acid on a dry weight basis [38]. In earlier studies, the compounds found in the fruit were incorrectly recognized as citric acid, however, subsequent studies have demonstrated that the main acid in the *G. cambogia* fruit is hydroxycitric acid [38].

In the late 1960s, hydroxycitric acid was first identified as a potent competitive inhibitor of the extramitochondrial enzyme ATP-citrate lyase [39]. This enzyme is involved in the initial steps of cholesterol and fatty acid biosynthesis. As a result, hydroxycitric acid causes a reduction in the transformation of citrate into aceyl CoA, a step essential for fatty acid formation in the liver. Furthermore, hepatic glycogen accumulates in the presence of hydroxycitric acid, this metabolic change may in turn cause the activation of glucoreceptors and thus result in the feeling of satiety or reduced appetite [40, 41]. In addition, hydroxycitric acid has been found to increase the release of [3H]-5-hydroxytryptamine (serotonin) from isolated brain cortical slices in rats [42]. As it is known that serotonin is involved in regulating eating behaviour and body weight control, it has been hypothesized that a mechanism of curbing appetite promoted by hydroxycitric acid administration may be facilitated by this neurotransmitter [43].

Another potential mechanism of hydroxycitric acid may be that by limiting acetyl CoA availability, the level of malonyl CoA is depressed consequently lowering the negative feedback on carnitineacyl transferase (Fig. 2) [44]. In turn, this causes enhanced lipid transport into the mitochondria and oxidation becomes efficient with the formation of ketone bodies. Ketones are recognized as appetite suppressants, although there is limited evidence to suggest that ketosis is related to hunger levels (Fig. 2) [45].

In studies using rats, a satiety effect followed by weight reduction has been demonstrated with hydroxycitric acid administration [41, 46–48]. In addition, a human trial conducted by Preuss et al. [49] found a significant reduction in food intake, BMI and body weight following administration of hydroxycitric acid. However, other human trials have failed to show a satiety effect following supplementation of the extract compared to the placebo [44, 50–53]). A more bioavailable, water soluble calcium-potassium salt of hydroxycitric acid was administered in the study by Preuss et al. [49]. Therefore, further investigations on the administration method and dosage are needed for future clinical trials. Overall, the evidence for *G. cambogia* in human clinical trials is not convincing.

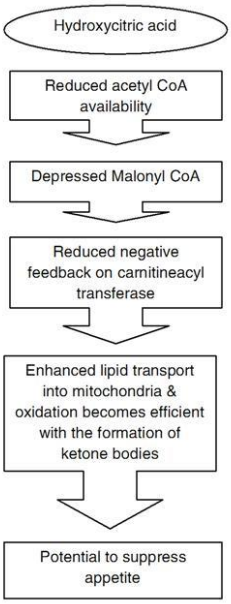

Fig. 2 Potential mechanisms of hydroxycitric acid in appetite suppression

Phaseolus vulgaris

P. vulgaris is a popular ingredient used in weight loss products. Red kidney beans *(P. vulgaris)* are produced by a herbaceous plant that belongs to the leguminosae family. The *P. vulgaris* genus includes all species of legume seeds which are typically referred as common beans. The common bean is the most important food legume and major source of protein in developing countries such as Latin America [54]. Archaeological studies have found that these beans originated in America, including Southern United States, the

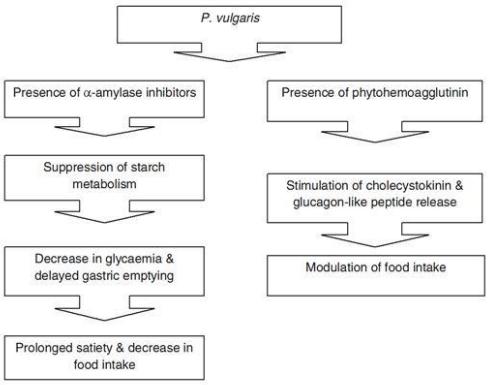

Fig. 3 Proposed mechanisms of *P. vulgaris* in appetite suppression

Northern part of South America, Central America and Mexico [55]. Specifically, the species *P. vulgaris* was introduced into Europe in the XVI century and has now become an imperative crop in several regions worldwide such as Latin American and African countries [56, 57]. Black and white varieties are also available, however they are not used as often. To consumers, color and size are important characteristics for quality. The seed color of the beans is reflective of the concentration and presence of polyphenolic compounds including anthocyanins, flavonol glycosides and proanthocyanidins (condensed tannins) [58]. The most abundant group of flavonoids in beans are the proanthocyanidins [59]. The common bean is considered a functional food as it has many phytochemicals including polyphenolic compounds, lectins, phytic acid, dietary fibers, unsaturated fatty acids, trypsin inhibitors in addition to proanthocyanidins [60].

A reduction in food intake following administration of *P. vulgaris* extract is attributed to the presence of two lectins including α-amylase inhibitors and phytohemoagglutinin [61]. Pancreatic enzyme α-amylase inhibition results in the suppression of starch metabolism which consequently causes a decrease in glycemia [62] and gastric emptying is delayed which in turn prolongs satiety and thus food intake is reduced (Fig. 3) [63, 64]. Furthermore, the lectin phytohemoagglutinin binds to the stomach epithelial cells and intestinal brush border of the small intestine, cecum and colon, which results in the stimulation of cholecystokinin and glucagon-like peptide release which are involved in the modulation of food intake (Fig. 3) [65–67].

Animal trials have shown that administration of *P. vulgaris* significantly reduces food intake, body mass and lipid accumulation in obese and lean animals [62, 68–78]. In addition, studies have shown that *P. vulgaris* extract may potentially reduce the consumption of highly palatable foods such as butter cookies and chocolate-flavoured beverages [56]. This suggests that *P. vulgaris* may have suppressive effects on palatable foods. There are limited studies on *P. vulgaris* in humans. A study conducted by Udani et al. [79] found that after eight weeks of *P. vulgaris* supplementation, there was no change in energy intake, appetite control, hunger, body weight or body fat in the experimental group compared to placebo. Hunger, energy and appetite control were measured via the 10-point Likert scale. No adverse events occurred due to the active products. A major limitation to this study was a small sample size with only 27 participants completing the trial. Further studies with a larger sample size would be needed to identify a statistically significant result.

Concluding Remarks

Botanical species including crude extracts and isolated compounds from plants have been shown to provide potentially promising therapeutic effects including appetite control and body fat reduction in animal studies. However, there is lack of evidence to support that these supplements are effective in suppressing appetite in humans. Many of these crude extracts and compounds need to be further investigated to define the magnitude of the effects, optimal dosage, mechanisms of action, long term safety and potential side effects. Furthermore, even though some natural supplements have been proven to be effective in short term trials, the chronic use of these compounds is still yet to be determined.

This review focused on specific chemical constituents that influence appetite, although it is necessary to mention that there are many more compounds at present under exploration. In addition, it is important to state that even though several medicinal plant extracts are obtainable over the counter (non-prescription weight loss products), many scientifically tested products may not have proven to recent work. Enhanced understanding and evidence-based research on these compounds and crude extracts will guide further rigorous investigations as well as new investigations of natural plant based products that provide the most effective benefits to society.

Conflict of Interest The authors declare that there is no conflict of interest.

References

1. Celleno L, Tolaini MV, D'Amore A, Perricone NV, Preuss HG (2007) A dietary supplement containing standardized *Phaseolus vulgaris* extract influences body composition of overweight men and women. Int J Med Sci 4(1):45–52
2. Santos AP, Rogero MM, Bastos DH (2010) Edible plants, their secondary metabolites and antiobesogenic potential. Recent Pat Food Nutr Agric 2(3):195–212
3. Rains TM, Agarwal S, Maki KC (2011) Antiobesity effects of green tea catechins: a mechanistic review. J Nutr Biochem 22(1):1–7. doi:10.1016/j.jnutbio.2010.06.006
4. Wolfram S, Wang Y, Thielecke F (2006) Anti-obesity effects of green tea: from bedside to bench. Mol Nutr Food Res 50(2):176–187. doi:10.1002/mnfr.200500102
5. Scharrer E (1999) Control of food intake by fatty acid oxidation and ketogenesis. Nutrition 15(9):704–714
6. Friedman MI (2007) Obesity and the hepatic control of feeding behavior. Drug News Perspect 20(9):573–578. doi:10.1358/dnp.2007.20.9.1162243
7. Kamphuis MM, Mela DJ, Westerterp-Plantenga MS (2003) Diacylglycerols affect substrate oxidation and appetite in humans. Am J Clin Nutr 77(5):1133–1139
8. Murase T, Nagasawa A, Suzuki J, Hase T, Tokimitsu I (2002) Beneficial effects of tea catechins on diet-induced obesity: stimulation of lipid catabolism in the liver. Int J Obes Relat Metab Disord 26(11):1459–1464. doi:10.1038/sj.ijo.0802141
9. Kao YH, Hiipakka RA, Liao S (2000) Modulation of endocrine systems and food intake by green tea epigallocatechin gallate. Endocrinology 141(3):980–987

10. Klaus S, Pultz S, Thone-Reineke C, Wolfram S (2005) Epigallocatechin gallate attenuates diet-induced obesity in mice by decreasing energy absorption and increasing fat oxidation. Int J Obes (Lond) 29(6):615–623. doi:10.1038/sj.ijo.0802926
11. Choo JJ (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
12. Hasegawa N, Yamda N, Mori M (2003) Powdered green tea has antilipogenic effect on Zucker rats fed a high-fat diet. Phytother Res 17(5):477–480. doi:10.1002/ptr.1177
13. 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
14. Bose M, Lambert JD, Ju J, Reuhl KR, Shapses SA, Yang CS (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
15. Ito Y, Ichikawa T, Morohoshi Y, Nakamura T, Saegusa Y, Ishihara K (2008) Effect of tea catechins on body fat accumulation in rats fed a normal diet. Biomed Res 29(1):27–32
16. Kovacs EM, Lejeune MP, Nijs I, Westerterp-Plantenga MS (2004) Effects of green tea on weight maintenance after body-weight loss. Br J Nutr 91(3):431–437. doi:10.1079/BJN20041061
17. Nagao T, Hase T, 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. doi:10.1038/oby.2007.176
18. Maki KC, Reeves MS, Farmer M, Yasunaga K, Matsuo N, Katsuragi Y, Komikado M, Tokimitsu I, Wilder D, Jones F, Blumberg JB, Cartwright Y (2009) Green tea catechin consumption enhances exercise-induced abdominal fat loss in overweight and obese adults. J Nutr 139(2):264–270. doi:10.3945/jn.108.098293
19. Wang H, Wen Y, Du Y, Yan X, Guo H, Rycroft JA, Boon N, Kovacs EM, Mela DJ (2010) Effects of catechin enriched green tea on body composition. Obesity (Silver Spring) 18(4):773–779. doi:10.1038/oby.2009.256
20. Gregersen NT, Bitz C, Krog-Mikkelsen I, Hels O, Kovacs EM, Rycroft JA, Frandsen E, Mela DJ, Astrup A (2009) Effect of moderate intakes of different tea catechins and caffeine on acute measures of energy metabolism under sedentary conditions. Br J Nutr 102(8):1187–1194. doi:10.1017/S0007114509371779
21. Auvichayapat P, Prapochanung M, Tunkamnerdthai O, Sripanidkulchai BO, Auvichayapat N, Thinkhamrop B, Kunhasura S, Wongpratoom S, Sinawat S, Hongprapas P (2008) Effectiveness of green tea on weight reduction in obese Thais: a randomized, controlled trial. Physiol Behav 93(3):486–491. doi:10.1016/j.physbeh.2007.10.009
22. Kuriyan R, Raj T, Srinivas SK, Vaz M, Rajendran R, Kurpad AV (2007) Effect of Caralluma fimbriata extract on appetite, food intake and anthropometry in adult Indian men and women. Appetite 48(3):338–344. doi:10.1016/j.appet.2006.09.013
23. Kunert O, Rao VG, Babu GS, Sujatha P, Sivagamy M, Anuradha S, Rao BV, Kumar BR, Alex RM, Schuhly W, Kuhnelt D, Rao GV, Rao AV (2008) Pregnane glycosides from Caralluma adscendens var. fimbriata. Chem Biodivers 5(2):239–250. doi:10.1002/cbdv.200890021
24. Gardiner JV, Kong WM, Ward H, Murphy KG, Dhillo WS, Bloom SR (2005) AAV mediated expression of anti-sense neuropeptide Y cRNA in the arcuate nucleus of rats results in decreased weight gain and food intake. Biochem Biophys Res Commun 327(4):1088–1093. doi:10.1016/j.bbrc.2004.12.113
25. Kamalakkannan S, Rajendran R, Venkatesh RV, Clayton P, Akbarsha MA (2010) Antiobesogenic and antiatherosclerotic properties of caralluma fimbriata extract. J Nutr Metab 2010:285301. doi:10.1155/2010/285301
26. Astell KJ, Mathai LM, McAinach AJ, Stathis CG, Su XQ (2013) A pilot study investigating the effect of Caralluma fimbriata extract on the risk factors of metabolic syndrome in overweight and obese subjects: a randomised controlled clinical trial. Complement Ther Med. doi:10.1016/j.ctim.2013.01.004
27. Dutt HC, Singh S, Avula B, Khan IA, Bedi YS (2012) Pharmacological review of Caralluma R.Br. with special reference to appetite suppression and anti-obesity. J Med Food 15(2):108–119. doi:10.1089/jmf.2010.1555
28. Pellati F, Benvenuti S, Melegari M, Firenzuoli F (2002) Determination of adrenergic agonists from extracts and herbal products of Citrus aurantium L. var. amara by LC. J Pharm Biomed Anal 29(6):1113–1119
29. Haaz S, Fontaine KR, Cutter G, Limdi N, Perumean-Chaney S, Allison DB (2006) Citrus aurantium and synephrine alkaloids in the treatment of overweight and obesity: an update. Obes Rev 7(1):79–88. doi:10.1111/j.1467-789X.2006.00195.x
30. Astrup A (2000) Thermogenic drugs as a strategy for treatment of obesity. Endocrine 13(2):207–212. doi:10.1385/ENDO:13:2:207
31. Stricker EM, Verbalis JG (1991) Caloric and noncaloric controls of food intake. Brain Res Bull 27(3–4):299–303
32. Yeh SY (1999) Comparative anorectic effects of metaraminol and phenylephrine in rats. Physiol Behav 68(1–2):227–234
33. Greenway F, de Jonge-Levitan L, Martin C, Roberts A, Grundy I, Parker C (2006) Dietary herbal supplements with phenylephrine for weight loss. J Med Food 9(4):572–578. doi:10.1089/jmf.2006.9.572
34. Litosch I, Hudson TH, Mills I, Li SY, Fain JN (1982) Forskolin as an activator of cyclic AMP accumulation and lipolysis in rat adipocytes. Mol Pharmacol 22(1):109–115
35. Godard MP, Johnson BA, Richmond SR (2005) Body composition and hormonal adaptations associated with forskolin consumption in overweight and obese men. Obes Res 13(8):1335–1343. doi:10.1038/oby.2005.162
36. Han LK, Morimoto C, Yu RH, Okuda H (2005) Effects of *Coleus forskohlii* on fat storage in ovariectomized rats. Yakugaku Zasshi 125(5):449–453
37. Henderson S, Magu B, Rasmussen C, Lancaster S, Kerksick C, Smith P, Melton C, Cowan P, Greenwood M, Earnest C, Almada A, Milnor P, Magrans T, Bowden R, Ounpraseuth S, Thomas A, Kreider RB (2005) Effects of *Coleus forskohlii* supplementation on body composition and hematological profiles in mildly overweight women. J Int Soc Sports Nutr 2:54–62. doi:10.1186/1550-2783-2-2-54
38. Soni MG, Burdock GA, Preuss HG, Stohs SJ, Ohia SE, Bagchi D (2004) Safety assessment of (-)-hydroxycitric acid and Super CitriMax, a novel calcium/potassium salt. Food Chem Toxicol 42(9):1513–1529. doi:10.1016/j.fct.2004.04.014
39. Watson JA, Fang M, Lowenstein JM (1969) Tricarballylate and hydroxycitrate: substrate and inhibitor of ATP: citrate oxaloacetate lyase. Arch Biochem Biophys 135(1):209–217
40. Triscari J, Sullivan AC (1984) Anti-obesity activity of a novel lipid synthesis inhibitor. Int J Obes 8(Suppl 1):227–239
41. Lowenstein JM (1971) Effect of (-)-hydroxycitrate on fatty acid synthesis by rat liver *in vivo*. J Biol Chem 246(3):629–632
42. Ohia SE, Awe SO, LeDay AM, Opere CA, Bagchi D (2001) Effect of hydroxycitric acid on serotonin release from isolated rat brain cortex. Res Commun Mol Pathol Pharmacol 109(3–4):210–216
43. Preuss HG, Rao CV, Garis R, Bramble JD, Ohia SE, Bagchi M, Bagchi D (2004) An overview of the safety and efficacy of a novel, natural(-)-hydroxycitric acid extract (HCA-SX) for weight management. J Med 35(1–6):33–48

44. Mattes RD, Bormann L (2000) Effects of (-)-hydroxycitric acid on appetitive variables. Physiol Behav 71(1–2):87–94
45. Baird IM, Parsons RL, Howard AN (1974) Clinical and metabolic studies of chemically defined diets in the management of obesity. Metabolism 23(7):645–657
46. Sullivan AC, Triscari J, Hamilton JG, Miller ON (1974) Effect of (-)-hydroxycitrate upon the accumulation of lipid in the rat. II. Appetite. Lipids 9(2):129–134
47. Sullivan AC, Hamilton JG, Miller ON, Wheatley VR (1972) Inhibition of lipogenesis in rat liver by (-)-hydroxycitrate. Arch Biochem Biophys 150(1):183–190
48. Sergio W (1988) A natural food, the Malabar tamarind, may be effective in the treatment of obesity. Med Hypotheses 27(1):39–40
49. Preuss HG, Bagchi D, Bagchi M, Rao CV, Dey DK, Satyanarayana S (2004) Effects of a natural extract of (-)-hydroxycitric acid (HCA-SX) and a combination of HCA-SX plus niacin-bound chromium and *Gymnema sylvestre* extract on weight loss. Diabetes Obes Metab 6(3):171–180. doi:10.1111/j.1462-8902.2004.00328.x
50. Vasques CA, Rossetto S, Halmenschlager G, Linden R, Heckler E, Fernandez MS, Alonso JL (2008) Evaluation of the pharmacotherapeutic efficacy of *Garcinia cambogia* plus *Amorphophallus konjac* for the treatment of obesity. Phytother Res 22(9):1135–1140. doi:10.1002/ptr.2323
51. Hackman RM, Havel PJ, Schwartz HJ, Rutledge JC, Watnik MR, Noceti EM, Stohs SJ, Stern JS, Keen CL (2006) Multinutrient supplement containing ephedra and caffeine causes weight loss and improves metabolic risk factors in obese women: a randomized controlled trial. Int J Obes (Lond) 30(10):1545–1556. doi:10.1038/sj.ijo.0803283
52. Kovacs EM, Westerterp-Plantenga MS, de Vries M, Brouns F, Saris WH (2001) Effects of 2-week ingestion of (-)-hydroxycitrate and (-)-hydroxycitrate combined with medium-chain triglycerides on satiety and food intake. Physiol Behav 74(4–5):543–549
53. Heymsfield SB, Allison DB, Vasselli JR, Pietrobelli A, Greenfield D, Nunez C (1998) *Garcinia cambogia* (hydroxycitric acid) as a potential antiobesity agent: a randomized controlled trial. JAMA 280(18):1596–1600
54. Landa-Habana L, Pina-Hernandez A, Agama-Acevedo E, Tovar J, Bello-Perez LA (2004) Effect of cooking procedures and storage on starch bioavailability in common beans (*Phaseolus vulgaris* L.). Plant Foods Hum Nutr 59(4):133–136
55. Sotelo A, Sousa H, Sanchez M (1995) Comparative study of the chemical composition of wild and cultivated beans (*Phaseolus vulgaris*). Plant Foods Hum Nutr 47(2):93–100
56. Carai MA, Fantini N, Loi B, Colombo G, Riva A, Morazzoni P (2009) Potential efficacy of preparations derived from *Phaseolus vulgaris* in the control of appetite, energy intake, and carbohydrate metabolism. Diabetes Metab Syndr Obes 2:145–153
57. Acevedo E, Velazquez-Coronado L, Bressani R (1994) Changes in dietary fiber content and its composition as affected by processing of black beans (*Phaseolus vulgaris*, Tamazulapa variety). Plant Foods Hum Nutr 46(2):139–145
58. Islam FM, Rengifo J, Redden RJ, Basford KE, Beebe SE (2003) Association between seed coat polyphenolics (tannins) and disease resistance in common bean. Plant Foods Hum Nutr 58(4):285–297
59. Beninger CW, Hosfield GL (2003) Antioxidant activity of extracts, condensed tannin fractions, and pure flavonoids from *Phaseolus vulgaris* L. seed coat color genotypes. J Agric Food Chem 51(27):7879–7883. doi:10.1021/jf0304324
60. Aparicio-Fernandez X, Reynoso-Camacho R, Castano-Tostado E, Garcia-Gasca T, Gonzalez de Mejia E, Guzman-Maldonado SH, Elizondo G, Yousef GG, Lila MA, Loarca-Pina G (2008) Antiradical capacity and induction of apoptosis on HeLa cells by a *Phaseolus vulgaris* extract. Plant Foods Hum Nutr 63(1):35–40. doi:10.1007/s11130-007-0066-4
61. Fantini N, Cabras C, Lobina C, Colombo G, Gessa GL, Riva A, Donzelli F, Morazzoni P, Bombardelli E, Carai MA (2009) Reducing effect of a *Phaseolus vulgaris* dry extract on food intake, body weight, and glycemia in rats. J Agric Food Chem 57(19):9316–9323. doi:10.1021/jf900711z
62. Tormo MA, Gil-Exojo I, Romero de Tejada A, Campillo JE (2004) Hypoglycaemic and anorexigenic activities of an alpha-amylase inhibitor from white kidney beans (*Phaseolus vulgaris*) in Wistar rats. Br J Nutr 92(5):785–790
63. Jain NK, Boivin M, Zinsmeister AR, DiMagno EP (1991) The ileum and carbohydrate-mediated feedback regulation of postprandial pancreaticobiliary secretion in normal humans. Pancreas 6(5):495–505
64. Jain NK, Boivin M, Zinsmeister AR, Brown ML, Malagelada JR, DiMagno EP (1989) Effect of ileal perfusion of carbohydrates and amylase inhibitor on gastrointestinal hormones and emptying. Gastroenterology 96(2):377–387
65. Herzig KH, Bardocz S, Grant G, Nustede R, Folsch UR, Pusztai A (1997) Red kidney bean lectin is a potent cholecystokinin releasing stimulus in the rat inducing pancreatic growth. Gut 41(3):333–338
66. Bardocz S, Grant G, Ewen SW, Duguid TJ, Brown DS, Englyst K, Pusztai A (1995) Reversible effect of phytohemagglutinin on the growth and metabolism of rat gastrointestinal tract. Gut 37(3):353–360
67. King TP, Pusztai A, Grant G, Slater D (1986) Immunogold localization of ingested kidney bean (*Phaseolus vulgaris*) lectins in epithelial cells of the rat small intestine. Histochem J 18(8):413–420
68. Pusztai A, Bardocz S, Ewen SW (2008) Uses of plant lectins in bioscience and biomedicine. Front Biosci 13:1130–1140
69. Tormo MA, Gil-Exojo I, Romero de Tejada A, Campillo JE (2006) White bean amylase inhibitor administered orally reduces glycemia in type 2 diabetic rats. Br J Nutr 96(3):539–544
70. Baintner K, Kiss P, Pfuller U, Bardocz S, Pusztai A (2003) Effect of orally and intraperitoneally administered plant lectins on food consumption of rats. Acta Physiol Hung 90(2):97–107. doi:10.1556/APhysiol.90.2003.2.2
71. Pusztai A, Grant G, Buchan WC, Bardocz S, de Carvalho AF, Ewen SW (1998) Lipid accumulation in obese Zucker rats is reduced by inclusion of raw kidney bean (*Phaseolus vulgaris*) in the diet. Br J Nutr 79(2):213–221
72. Pusztai A, Grant G, Duguid T, Brown DS, Peumans WJ, Van Damme EJ, Bardocz S (1995) Inhibition of starch digestion by alpha-amylase inhibitor reduces the efficiency of utilization of dietary proteins and lipids and retards the growth of rats. J Nutr 125(6):1554–1562
73. Grant G, Dorward PM, Buchan WC, Armour JC, Pusztai A (1995) Consumption of diets containing raw soya beans (*Glycine max*), kidney beans (*Phaseolus vulgaris*), cowpeas (*Vigna unguiculata*) or lupin seeds (*Lupinus angustifolius*) by rats for up to 700 days: effects on body composition and organ weights. Br J Nutr 73(1):17–29
74. Grant G, Dorward PM, Pusztai A (1993) Pancreatic enlargement is evident in rats fed diets containing raw soybeans (*Glycine max*) or cowpeas (*Vigna unguiculata*) for 800 days but not in those fed diets based on kidney beans (*Phaseolus vulgaris*) or lupinseed (Lupinus angustifolius). J Nutr 123(12):2207–2215
75. Donatucci DA, Liener IE, Gross CJ (1987) Binding of navy bean (*Phaseolus vulgaris*) lectin to the intestinal cells of the rat and its effect on the absorption of glucose. J Nutr 117(12):2154–2160
76. Maranesi M, Barzanti V, Biagi PL, Carenini G, Gentili P (1984) Nutritional studies on anti-alpha-amylase: II) Lipid metabolism

investigation: fatty acid composition of organs and tissues. Acta Vitaminol Enzymol 6(4):347–353
77. Maranesi M, Carenini G, Gentili P (1984) Nutritional studies on anti alpha-amylase: I) Influence on the growth rate, blood picture and biochemistry and histological parameters in rats. Acta Vitaminol Enzymol 6(4):259–269
78. Kakade ML, Evans RJ (1966) Growth inhibition of rats fed raw navy beans (*Phaseolus vulgaris*). J Nutr 90(2):191–198
79. Udani J, Hardy M, Madsen DC (2004) Blocking carbohydrate absorption and weight loss: a clinical trial using phase 2 brand proprietary fractionated white bean extract. Altern Med Rev 9(1):63–69

Appendix 2 - Plant extracts with appetite suppressing properties for body weight control: A systematic review of double blind randomized controlled clinical trials

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Plant extracts with appetite suppressing properties for body weight control: A systematic review of double blind randomized controlled clinical trials

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KEYWORDS
Plant extracts;
Appetite;
Food intake;
Body weight

Summary

Overview: As obesity has reached epidemic proportions, the management of this global disease is of clinical importance. The availability and popularity of natural dietary supplements for the treatment of obesity has risen dramatically in recent years.

Aims: The aim of this paper was to assess the current evidence of commonly available natural supplements used to suppress appetite for obesity control and management in humans using a systematic search of clinical trials meeting an acceptable standard of evidence.

Methods: The electronic databases PubMed, Web of Science, Google Scholar, ScienceDirect, and MEDLINE with full text (via EBSCOHost) were accessed during late 2012 for randomized controlled clinical trials (RCTs) using natural plant extracts as interventions to treat obesity through appetite regulation. A quality analysis using a purpose-designed scale and an estimation of effect size, where data were available, was also calculated. The inclusion criteria included the following: sample participants classified as overweight or obese adults (aged 18–65 years), randomized, double blind, controlled design, suitable placebo/control intervention, sample size >20, duration of intervention >2 weeks, have measurable outcomes on appetite or food intake and anthropometry, and full paper in English.

Results: There were 14 studies that met the inclusion criteria. The findings from published double blind RCTs revealed mostly inconclusive evidence that plant extracts are effective in reducing body weight through appetite suppression. *Caralluma fimbriata* extract and a combination supplement containing *Garcinia cambogia* plus *Gymnema sylvestre* were the only exceptions.

Conclusion: According to the findings from this systematic review, the evidence is not convincing in demonstrating that most dietary supplements used as appetite suppressants for weight loss in the treatment of obesity are effective and safe. A balance between conclusive findings by double blind RCTs and advertisement is required to avoid safety concerns and dissatisfaction from consumers.

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Contents

Background

The prevalence of obesity is reaching epidemic proportions worldwide, which is associated with several co-morbidities such as type 2 diabetes, dyslipidemia, degenerative arthritis, obstructive sleep apnea, hypertension and cardiovascular disease.[1] Fortunately, there is strong evidence that modest body weight loss of 5—10% significantly reduces the risk of these co-morbidities.[2] There are a variety of effective options for weight loss in the treatment of overweight and obesity which include dietary therapies, altering physical activity, behavioural techniques, pharmacotherapy, surgery and a combination of these strategies.[2] The first-line of therapy for the management of obesity has the least risk which consists of lifestyle changes including diet, exercise and behavioural modification. The second-line of therapy for obesity treatment is pharmacotherapy, which is often recommended when lifestyle modification is ineffective in producing sufficient weight loss. The last approach in extreme cases of morbid obesity is through surgical therapy. Surgical treatment is an option for a limited number of patients with clinically severe or morbid obesity (BMI > 40 or >35 with comorbid conditions) and is reserved for those who are suffering from the complications associated with extreme obesity or are unresponsive to nonsurgical treatment.[3] Due to the difficulty in maintaining sustained lifestyle changes, potential complications of surgery and accompaniment of serious adverse effects associated with pharmacotherapy, it is not surprising that the general public frequently turn to easily obtainable over the counter proprietary weight loss products such as herbal products, nutritional supplements and meal replacements. Pharmacognosy research including medical ethnobotany, ethnopharmacology, and phytotherapy studies as well as rigorous RCTs are yet to be carried out on many of these products and in reality, marketing takes priority over the safety and efficacy of many weight loss products. The findings of a multi-state survey conducted in the US revealed that 7% of adults used non-prescription/over the counter weight loss supplements, with a greater proportion of use among young obese women.[4] In addition, retail sales of weight loss supplements were estimated to be greater than $1.3 billion in 2001.[5]

Plant extracts have been used for many centuries in the Eastern world, however the use of these extracts have recently become increasingly prevalent around the world. Several chemical constituents isolated from plants and crude extracts have been found to prevent diet induced obesity and significantly reduce body weight in the treatment of obesity. Due to the prevalent use of plant extracts, evidence is required to support claims of efficacy. Previous publications have explored anti-obesity agents for weight loss, however to date no systematic review has been conducted on plant extracts that elicit appetite suppression properties, assessing the quality of studies as well as assessing the methodology, dosage, duration of intervention and the strength of their clinical effects. This paper provides details in biochemical characterization of bioactive compounds from plant extracts, methods used for assessing appetite, and provides a toxicological evaluation and clinical evaluation including efficacy of plants extracts in RCTs. Thus the purpose of this paper is to present a systematic review of plant extracts possessing appetite suppressing properties for obesity treatment.

Methods

The electronic databases PubMed, ScienceDirect, Web of Science, Google Scholar, and MEDLINE with full text (via EBSCOHost) were accessed up to December 2012 (see Fig. 1 for systematic flowchart). The databases were searched using anti-obesity search terms in combination with specific interventions using plant extracts (see Appendix 1 for intervention search term list). Papers that met the inclusion criteria were human RCTs of acceptable methodological rigour.

The inclusion criteria included:

1. Sample participants classified as overweight or obese adults (aged 18—65 years)
2. Randomized, double blind, controlled design
3. Suitable placebo/control intervention
4. Sample size >20
5. Duration of intervention >2 weeks
6. Have measurable outcomes on appetite or food intake and anthropometry
7. Full paper in English

Studies that involved a combination treatment were considered acceptable. All other papers that did not meet these criteria as well as systematic reviews and meta-analyses were excluded. All studies were selected according to defined criteria and data were validated and extracted in

a systematic manner. All papers were analyzed for methodological quality using a modified augmented Jadad scale[6] that includes three primary quality factors – randomization, blinding and reported withdrawals. The modified augmented version assesses other methodological factors including exclusion criteria, intervention used, control used, and data reporting to provide a quality total out of 10. The modified criteria are noted in italics. Effect sizes were reported in all placebo-controlled studies where the results were significant (small clinical effect = 0.2, medium clinical effect = 0.5, large clinical effect = 0.8). The effect size of the clinical trials was calculated by the authors. The effect size was calculated as Cohen's *d*[7] by firstly calculating the effect size separately within the active and control group (taking the difference between baseline and post-intervention means, divided by the within-group standard deviation at baseline), and secondly subtracting the effect size of the control group from that of the active group.

Modified Jadad questionnaire:

1. Was the study described as randomized?
2. Was the randomization protocol detailed and appropriate?
3. Was the study described as double blind?
4. Was the blinding process detailed and appropriate?
5. Did the study have a control group?
6. Was the control detailed and appropriate?
7. Was there an appropriate exclusion criteria?
8. Was the intervention used at a therapeutic dose?
9. Was there a description of withdrawals and dropouts?
10. Were the data clearly and adequately reported?

Yes = 1 point; No = 0 point; Total/10

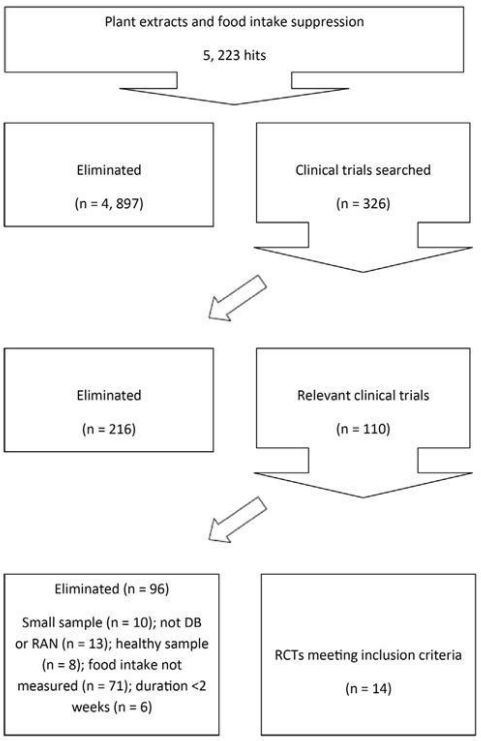

Figure 1 Systematic review flowchart.

Results

Overview of results

Out of 5223 located potential studies in the area of plant extracts used for appetite suppression in obesity treatment, 326 were found to be RCTs. Two hundred and sixteen (216) were eliminated, commonly due to irrelevance, methodological weakness (small sample size, not controlled, double blind or randomized), sample with BMI within healthy range, studies that did not measure food intake and duration of intervention <2 weeks. This left fourteen clinical trials for inclusion. Twenty-seven potential appetite suppressant plant extracts were identified in this literature search with only ten specific interventions using mono plant extracts or in combination met the criteria for inclusion: *Amorphophallus Konjac C.* (Konjac glucomannon); *Camellia sinensis* (Green tea; *Caralluma fimbriata* (Slimaluma); *Garcinia cambogia* (Hydroxycitric acid); *G. cambogia* + *Gymnema sylvestre*, *G. cambogia* + *A. Konjac*; *G. cambogia* + *C. sinensis* + *Ephedra sinica* + *G. sylvestre*; *Irvingia gabonensis*; *Trigonella foenum-graecum L.* (Fenugreek seed extract); *Hoodia gordonii*; *Phaseolus vulgaris*. Their key details are summarized in Table 1.

Plant extracts

Fourteen studies using plant extracts met the criteria for inclusion. These had an overall quality rating of 7.9 (range 6–10), with 9 RCTs revealing a quality rating of 8 or greater out of 10 (Table 1). The average sample size was 72 (range 21–240), and the average duration of the study was 10.6 weeks (range 2–39). The effect size was only calculated in 3 RCTs as the other studies had non-significant findings or the effect size calculation was not possible due to limited data being provided.

The plant extract with the most research was found to be *G. cambogia*. In our systematic review of the literature, five RCTs with *G. cambogia* as an ingredient in the test product were located that met the criteria for inclusion, with only one producing significant positive results on the main primary outcome measure, i.e. appetite.[8] The study by Preuss et al.[8] was conducted using a highly bioavailable, water soluble calcium–potassium salt of HCA-SX (60% HCA). In addition, the supplements were administered on an empty stomach at best 30–60 min before meals to enhance bioavailability. Interestingly, the available sources of HCA currently on the market are a calcium salt which is considered poorly soluble (50% soluble in water), allowing for compromised absorption. In comparison to the other four

Table 1 Evidence of plant extract interventions with RCTs in the treatment of obesity via appetite or food intake suppression.

Intervention	First author	Methodology	Duration (weeks)	Result	Effect size	Quality/10
A. konjac	Chen (2003)[20]	DB, RAN, PC, CO, KGM ($n=10$; 0.5 g/day) or placebo ($n=12$; 0.5 g/day)	4	No significant difference was found in KGM group for BW and energy intake compared to placebo	Non-significant data provided	8
C. sinensis	Maki (2009)[13]	DB, RAN, PC, catechin beverage ($n=56$; 500 ml/day) or control ($n=51$; 500 ml/day)	12	No significant difference was found in Catechin group for BW and energy intake compared to placebo	Non-significant data provided	9
	Kovacs (2004)[15]	DB, RAN, PC, Green tea ($n=51$; 450 mg/day) or placebo ($n=53$; 450 mg/day)	13	No significant difference was found in the Green tea group for hunger, satiety and BW compared to placebo	Non-significant data provided	8
(water extract)	Nagao (2007)[14]	DB, RAN, PC, PAR MC, catechin ($n=123$; 340 ml/day) or control ($n=117$; 340 ml/day)	12	No significant difference was found in Catechin group for BW, energy intake and fat intake compared to placebo	Non-significant data provided	9
C. fimbriata (40% ethanol extract)	Kuriyan (2007)[16]	DB, RAN, PC, *Caralluma fimbriata* ($n=25$; 1 g/day) or placebo ($n=25$; 1 g/day)	8.5	Significant reduction in hunger levels and WC in *Caralluma* group compared to placebo	WC: 0.18; Hunger levels: 0.82	8
G. cambogia	Mattes (2000)[12]	DB, RAN, PC, PAR, *Garcinia cambogia* ($n=42$; 2.4 g/day) or placebo ($n=47$; 2.4 g/day)	12	No significant difference found in *Garcinia cambogia* group in appetite and energy intake compared to placebo Significant difference was found in *Garcinia cambogia* group in BW and waist circumference	Non-significant data provided Mean ± SD values not provided	6

	Kovacs (2001)[11]	DB, RAN, PC, CO, ($n = 21$), HCA + MCT (3.4 g/day), HCA (3.4/day) or placebo (3.4 g/day)	2	No significant difference found in *Garcinia cambogia* group in appetite, energy intake, eating behaviour, mood and BW compared to placebo	Non-significant data provided	6
Combination therapy: *G. cambogia* + *G. sylvestre*	Preuss (2004)[8]	DB, RAN, PC, HCA-SX, ($n = 20$; 14 g/day), HCA-SX + NBC + GSE ($n = 20$; 14 g HCA, 12 mg NBC, 1.2 g GSE) or placebo ($n = 20$)	8	Significant increase was found in HCA-SX group and HCA-SX + NBC + GSE group in remaining food on the plate (appetite) compared to placebo Significant reduction was found in HCA-SX group and HCA-SX + NBC + GSE group in body weight compared to placebo	HCA-SX: BW: 0.61; Appetite: 2.79 HCA-SX + NBC + GSE: BW: 0.8; Appetite: 12.6	7
Combination Therapy: *G. cambogia* + *A. konjac*	Vasques (2008)[9]	DB, RAN, PC, ($n = 32$; HCA: 2.4 g/day, KGM: 1.5 g/day) or placebo ($n = 26$; 3.9 g/day)	12	No significant difference was found in HCA + KGM group for BW and food intake compared to placebo	Non-significant data provided	6
Combination Therapy: *G. cambogia* + *Green tea extract* + *E. sinica* + *G. sylvestre*	Hackman (2006)[10]	DB, RAN, PC, treatment ($n = 19$; 40 mg/day ephedra, 100 mg/day caffeine) or placebo ($n = 23$)	39	No significant difference was found in treatment group for energy intake compared to placebo Significant difference was found in treatment group for weight loss compared to placebo	Non-significant data provided Mean ± SD values not provided	10

Table 1 (*Continued*)

Intervention	First author	Methodology	Duration (weeks)	Result	Effect size	Quality/10
I. gabonensis	Ngondi (2009)[18]	DB, RAN, PC, IGOB131 ($n = 52$; 300 mg/day) or placebo ($n = 50$; 300 mg/day)	10	No significant difference was found in IGOB131 group for food intake compared to placebo Significant difference was found in IGOB131 group for BW, WC and fat % compared to placebo	Non-significant data provided IGOB131: BW: 1.34; WC: 2.21; Fat %: 0.57	8
T. foenum-graecum L. (ethanol extract)	Chevassus (2010)[21]	DB, RAN, PC, PAR, fenugreek seed extract ($n = 19$; 1176 mg/day) or placebo ($n = 20$; 1176 mg/day)	6	No significant difference was found in fenugreek group for appetite, satiety scores, energy intake and BW compared to placebo	Non-significant data provided	7
H. gordonii (methanol extract)	Blom (2011)[17]	DB, RAN, PC, PAR, HgPE-formula ($n = 20$; HgPE 1110 mg/day) or placebo ($n = 22$)	2.1	No significant difference was found in Hoodia gordonii group for energy intake, BW and fat % compared to placebo	Non-significant data provided	10
P. vulgaris	Udani (2004)[19]	DB, RAN, PC, Phase 2 ($n = 14$; 3 g/day) or placebo ($n = 13$; 3 g/day)	8	No significant difference was found in Phase 2 group for energy level, appetite control, hunger, BW and fat % compared to placebo	Non-significant data provided	8

Effect size was based on modified Cohen's *d* (see Methodology section); Quality rating was based modified Jadad scale; DB: double blind; RAN: randomized; PC: placebo controlled; CO: crossover design; KGM: *Konjac glucomannan*; BW: body weight; PAR MC: parallel multicenter trial; WC: waist circumference; HCA: hydroxycitrate; MCT: medium-chain triglycerides; HCA-SX: super CitriMax; NBC: niacin-bound chromium; GSE: *Gymnema sylvestre*.

RCTs, this study was designed to better determine the effects of HCA-SX at a higher dose on satiety. Preuss et al.[8] demonstrated a statistically significant reduction in appetite (effect size: 2.79) and body weight (effect size: BW: 0.61) following supplementation of HCA-SX alone or in combination with NBC and GSE compared to the placebo. Appetite was estimated by weighing remaining food after each meal. No serious adverse effects were reported in any participants in this study. Laboratory safety markers were not reported in this study. The following minor adverse events were observed in group A (HCA-SX alone): leg cramps ($n = 1$), heartburn ($n = 2$), diarrhoea ($n = 4$), gas ($n = 4$), increased appetite ($n = 1$), headaches ($n = 7$), stomach burn ($n = 1$), skin rash ($n = 1$), menstrual bleeding ($n = 1$), and general weakness ($n = 2$). Although, it is worth noting that the adverse events were no significantly different from the placebo.

All other studies investigating the effects of hydroxycitric acid did not support a satiety effect in the treatment group compared to placebo.[9–12] Although the studies by Hackman et al.[10] and Mattes and Bormann[12] showed a significant reduction in daily energy intake in the experimental group following the intervention period compared to baseline data. Mattes and Bormann[12] assessed energy and nutrient intake using the Food Processor nutrient database and appetite was assessed via 24-h peak and nadir self-reported hunger ratings. In addition, a three factor eating questionnaire was used to assess dietary restraint and sensory function was evaluated using a nine-point category scale with end anchors of ''not at all (sweetness, saltiness, fat) and extremely (sweet, salty, high fat). There were no adverse events reported during the study period. In comparison, Hackman et al.[10] assessed dietary intake via a standardized self-administered food frequency questionnaire. The most common self-reported symptoms in the treatment group were: appetite decreased ($n = 22$), dizziness ($n = 5$), dry mouth ($n = 14$), energy increased ($n = 19$), fatigue ($n = 7$), headache ($n = 16$), insomnia ($n = 7$), nausea ($n = 7$), nervousness ($n = 13$), palpitations ($n = 13$).

Furthermore, these RCTs found a significant reduction in body weight following administration of *G. cambogia* or the multinutrient supplement containing *G. cambogia*, *Ephedra*, *G. sylvestre* & Green tea extract compared to placebo. However these studies failed to show any effect of HCA on appetite variables in the active treatment group compared to placebo. It is also worth noting, that safety parameters were not measured in the majority of these trials and the percentage biochemical characterization of *G. cambogia* extract was not described in any of the studies. Overall, the evidence for *G. cambogia* in isolation is not compelling.

Findings of studies on green tea (*C. sinensis*) extract on appetite, energy intake and body weight were negative.[10,13–15] Possible methodological flaws in these studies may have contributed to the negative results. For instances, the studies by Nagao et al.[14] and Kovacs et al.[15] did not limit tea and coffee consumption throughout the trial period. Subjects were investigated in a free-living condition, where there were no restrictions on caffeine consumption. Therefore, the magnitude of habitual caffeine consumption may have overruled the effectiveness of green tea supplementation. Nagao et al.[14] measured dietary intake via 5-day food diaries. Nagao et al.[14] confirmed the safety of the test beverage via analysis of biochemical and haematological parameters. While Kovacs et al.[15] measured attitudes towards eating via the three factor eating questionnaire and also measured appetite profile, hunger and satiety via the visual analogue scales method. No serious adverse events were reported in either study.[14,15]

There are many other potential limitations to these studies, such as it was observed that low caffeine consumers in the study by Kovacs et al.[15] had a stronger weight maintenance compared to high caffeine consumers, indicating that green tea supplementation may only be effective when habitual caffeine ingestion is low and that a much higher dose is required when habitual caffeine intake is high. Another possible explanation for failing to demonstrate the effect of green tea extract on food intake and body weight is a female predominant sample. The study by Hackman et al.[10] recruited healthy overweight pre-menopausal women and the majority of subjects in the study by Kovacs et al.[15] were women. It is possible that the impact of green tea on energy expenditure and fat oxidation is greater in men than in women, therefore a greater statistical power of male subjects may be required. Another limiting factor is the lack of reporting of characterization of *C. sinensis* extract in most of these studies.[10,13,15] Overall, these studies indicate that green tea supplementation is not effective in promoting weight loss through appetite suppression.

In a study conducted by Kuriyan et al.,[16] a significant reduction in hunger levels in the *C. fimbriata* group compared to placebo was observed, with a large clinical effect size of 0.82. The results of this study revealed a significant decline in waist circumference (3 cm in 2 months) in the active treatment group compared to placebo. The hunger levels of participants reduced by 20% following the administration period, which could account for an 8% decline in energy intake for the treatment group. *C. fimbriata* extract was made from the aerial plants of the plant with 40% aqueous alcohol. Dietary intake was assessed using a modified food frequency questionnaire and the visual analogue scales method was used to assess appetite sensations. Safety markers were not investigated in this study, however Kuriyan et al.[16] identified that in a previous animal study (Kurpad et al. unpublished) the LD50 of the extract did not reveal any toxicity and the LD50 was >5 g/kg. Also, there were only minor adverse events reported in the experimental group (24%), which include abdominal distention, flatulence, constipation and gastritis. The positive clinical results suggest that *C. fimbriata* extract may be used as an over-the-counter appetite suppressant for curbing appetite and reducing central adiposity.

A recent study by Blom et al.[17] investigating the effects of a 15 day repeated consumption of *H. gordonii* purified extract on satiety, ad libitum energy intake and body weight in healthy, overweight women appeared to be linked with significant adverse changes in several vital signs and laboratory parameters. The vital signs including systolic blood pressure, diastolic blood pressure and pulse rate were significantly higher in the treatment group compared to placebo. The safety laboratory results revealed that there was a significant increase in all bilirubin values and alkaline phosphatase in the treatment group. Common adverse events reported in the treatment group include disturbance of skin sensation ($n = 15$), headache ($n = 11$),

dizziness/giddiness ($n = 11$), nausea ($n = 12$), flushing ($n = 9$), vomiting ($n = 4$), fatigue ($n = 5$), flatulence/eructation/gas pain ($n = 2$), oedema ($n = 2$), injury/poisoning ($n = 11$), diseases of the musculoskeletal system and connective tissue ($n = 8$), diseases of the respiratory system ($n = 5$), supplemental classification of external causes of injury and poisoning ($n = 1$), and diseases of the blood and blood forming organs ($n = 4$).

H. gordonii was less well tolerated than the placebo and failed to show any significant effects on satiety, energy intake or body weight compared to placebo. Blom et al.[17] suggested that the dosage (1110 mg) of *H. gordonii* was not sufficient to induce a reduction in energy intake. The maximum average concentration of the steroidal glycoside H.g.-12 equivalents in blood plasma reached the target concentration of 100 ng/mL, however the inter-individual variation in maximum concentration was very large (range 15—355 ng/mL) with concentrations far greater than the concentration of 100 ng/mL. Due to the rapid absorption and elimination characteristics of H.g.-12 equivalents, resulting plasma concentration profiles may not be ideal to attain a reduction in energy intake. However, given the observed changes in clinical parameters and adverse symptoms reported at the current dose, the tolerability and safety of administration at a higher dose needs to be considered with caution.

The novel seed extract of the West African plant *I. gabonensis* (IGOB131) has been shown to significantly reduce body weight, waist size and body fat and improve metabolic parameters associated with insulin resistance compared to placebo in a randomized double-blind placebo controlled investigation,[18] although similar food intake habits and energy intake were observed in both experimental and placebo groups. Dietary intake was assessed via 3-day food diary records. The characterization of *I. gabonensis* extract was not described. Overall, IGOB131 was well tolerated, with minor adverse events which include headache ($n = 5$), sleep difficulty ($n = 6$), and intestinal flatulence ($n = 6$). These results suggest that IGOB131 is helpful in achieving weight loss although food intake was not affected.

A study conducted by Udani et al.[19] investigated the effect of the starch neutralizer brand bean extract referred to as *P. vulgaris* (Phase 2) on weight loss in a randomized double blind placebo controlled trial. This study did not find any clinically or statistically significant differences in energy intake, appetite control, hunger, body weight or body fat between the active treatment and placebo groups. Udani et al.[19] measured hunger, energy and appetite control via the 10-point Likert scales. The characterization of *P. vulgaris* extract was not described. There were minor adverse events reported in the treatment group including an increased incidence of tension headaches ($n = 1$). There were no significant differences in safety parameters across either group. A high dropout rate in this study may have limited the full potential for a treatment effect to occur. The effect size power calculation for this study was based on a previous study that achieved significant weight loss after 30 days of treatment. The high dropout rate was mainly due to requirement of multiple blood collections, the slow weight loss effect and high expectations of weight loss among subjects.

Supplementation of Konjac, *A. konjac* in overweight diabetic subjects did not result in a significant reduction in energy intake or body weight in a study conducted by Chen et al.[20] Two day food diaries were obtained from subjects which may not be considered as accurate or reliable for quantifying dietary intake, even though <15% variation between the two days was reported. Another methodological weakness is the lack of reporting of adverse events, safety parameters and the characterization of the extract. Furthermore, the relatively short intervention time of 4 weeks may not have allowed for clinical effects including energy intake reduction and body weight loss following Konjac supplementation to reach full potential.

The study conducted by Chevassus et al.[21] reported no significant change in spontaneous food intake during ad libitum meal test, body composition, appetite and satiety scores between fenugreek seed (*Trigonella foenum-graecum L.*) and placebo groups. Dietary intake was monitored via a 7-day food diary and appetite sensations were measured via the visual analogue scales method. Minor adverse effects were reported which included mild gastrointestinal symptoms. The sample size ($n = 39$) of this study may have been too small to reveal a significant difference for energy intake and weight loss, in addition to the short duration of 6 weeks. Furthermore, the inclusion and exclusion criteria were not described in the methodology section, therefore reducing the quality of the paper.

Discussion

The findings from published double blind RCTs revealed mostly inconclusive evidence that plant extracts are effective in reducing body weight through appetite suppression. *C. fimbriata* extract and a combination supplement containing *G. cambogia* plus *G. sylvestre* were the only exceptions. The relative lack of compelling evidence to suggest the effectiveness of appetite suppressant supplements in weight loss confirms the findings of previous literature reviews.[22]

The strength of this review is a rigorous systematic search criteria with coverage of all relevant studies across several databases. As discussed in the introduction, this is the first comprehensive systematic review to our knowledge on double blind RCTs investigating natural plant products used as appetite suppressants for body weight control. A further strength is the effect sizes were calculated to determine the clinical strength of the findings. We however acknowledge a few weaknesses with this review. While a systematic review of the literature is a gold standard methodological technique, such an approach may neglect studies that alter or may create a distorting effect to the landscape of the conclusions, arising from publication bias and location bias.[23,24] Therefore a limitation of this systematic review is the potential incompleteness of citation tracking, as it is conceivable that some studies may not have been found. In addition, there was substantial clinical trial literature, however the presence of basic methodological weaknesses led to the exclusion of many studies. Furthermore, in complementary medicine journals, positive findings may be overrepresented[25] and positive findings may be favoured at the expense of methodological quality.[26]

Lifestyle modification including reducing calorie intake and regular physical activity are the fundamental recommendations for successful weight loss and relative paucity

of evidence exists to support the efficacy of pharmacotherapy options. Poor compliance with demanding conventional weight loss programs which typically involve increasing energy expenditure and the popularity of complementary and alternative medicine have led to the marketing of non-prescription natural slimming aids. Although such preparations are popular and widely used, given the lack of supporting findings on efficacy, potential safety concerns including minor adverse side effects may impact on the risk-benefit balance against their use. Optimal and safe doses for body weight reduction are generally unknown, with several products containing combination formulas with unknown synergistic effects and nutrient interactions. For instance, there is no convincing data that *H. gordonii* is more effective than placebo and administration is associated with adverse events including episodes of nausea, emesis, disturbances of skin sensation and significant increases in blood pressure, heart rate, bilirubin and alkaline phosphatase.[17]

There were many confounding methodological flaws in many of the double blind RCTs, therefore reducing the quality of the papers and limiting the full potential of the effectiveness of the intervention. Methodological weaknesses observed include: unavailable data on biochemical characterization of extracts,[8,12,13,15,18–20] gender dominant,[10,15] small sample size[21] which may increase the possibility of spurious or false positive results and short intervention duration[18,20,21] which may make it difficult to assess the efficacy and safety of these supplements as an appetite suppressing agent on the medium to long term. Other methodological flaws include: adverse events experienced,[8,16–18,21] high dropout rates,[19] no restriction on caffeine consumption[14,15] and potential inadequate dosage of the active ingredient.[10,12,17]

The majority of RCTs identified from our search were not clear on the blinding process including how blinding was conducted and who was blinded to group assignment. The method of how randomization was carried out was also insufficient in most cases. The method used to generate the random allocation sequence, type of randomization and mechanism used to implement and conceal the random allocation sequence was not described in the majority of studies. None of the studies provided information about the monitoring of patient/staff blinding or analysis of patient/staff blinding. Information on the composition of the placebo capsule in many studies was also not provided. In addition the method of how the sample size was determined in most studies was not identified. Overall the lack of information reported in these RCTs casts doubt on the internal validity of these trials. Future research should focus on more clinical trials using double blind RCT methodology in line with the CONSORT guidelines.[27] This will ensure the validity and applicability of study results, in addition to longer intervention periods and employing adequate sample sizes. Long term efficacy, appropriate dosage and safety should also be considered in the methodology.

Conclusion

According to the findings from this systematic review, the evidence is not convincing in demonstrating that plant extracts used as appetite suppressants for weight loss in the treatment of obesity are effective and safe. Although some plant extracts have shown promising results in the short term, there is still a need for longer duration clinical trials to ultimately verify the traditional claims made that these plant extracts are effective in reducing energy intake through appetite suppression mechanisms. Despite the insufficient findings for safety and efficacy, several natural appetite suppressants are available as non-prescription herbal preparations. A major question to be addressed is whether the effect is achieved at an appropriate and safe dose. In addition, a balance between conclusive findings by double blind RCTs and advertisement is required to avoid safety concerns and dissatisfaction from consumers. Therefore, RCTs should be directed towards carefully evaluating the pharmacokinetics, bioavailability, efficacy, adverse effects and toxicity of bioactive compounds and their formulations, i.e. extracts.

Conflict of interest

The authors declare no conflict of interest.

Appendix 1. Intervention search terms

Outcome measure	Major interventions	Specific interventions (appetite suppressants)
Obesity	Nutritional medicine	*C. fimbriata*
Weight loss	Nutraceutical medicine	*H. gordonii*
Appetite	Ayuredic medicine	*Panax ginseng*
Energy intake	Herbal medicine	*G. cambogia*
Food intake	Natural medicine	*C. sinesis*
	Complementary medicine	*Coix lachrymajobi var mayeun*
	Plant extracts	*P. vulgaris*
	Medicinal plants	*Catha edulis*
	Crude plant extracts	*Citrus aurantium*
	Natural products	*G. sylvestre*
		Gyeongshingangjeehwan
		Green coffee bean
		Cyamopsis tetragonolobus
		Capsicum annuum
		Punica grantum L.
		A. konjac
		Benincasa hispida
		Mitragyna speciosa
		Cissus quadrangularis
		Ephedra sinica
		Robinia pseudoaccacia
		Sunflower oil
		Flaxseed dietary fibre
		Potein
		Evodiae fructus
		Eucommia
		Ilex paraguariensis

References

1. World Health Organization. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. *World Health Organization Technical Report Series* 2000;**894**(i—xii):1—253.
2. National Institutes of Health. Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults: the evidence report. *Obesity Research* 1998;**6**(Suppl. 2):51S—209S.
3. Fisher BL, Schauer P. Medical and surgical options in the treatment of severe obesity. *American Journal of Surgery* 2002;**184**:9S—16S.
4. Blanck HM, Khan LK, Serdula MK. Use of nonprescription weight loss products: results from a multistate survey. *Journal of the American Medical Asssociation* 2001;**286**:930—5.
5. Saper RB, Eisenberg DM, Phillips RS. Common dietary supplements for weight loss. *American Family Physician* 2004;**70**:1731—8.
6. Jadad AMA, Carroll D, Jenkinson D, Gavaghan D, McQuay H. Assessing the quality of reports of randomised clinical trials: is blinding necessary? *Controlled Clinical Trials* 1996;**17**:1—12.
7. Cohen J. *Statistical power analyses for the behavioral sciences*. 2nd ed. Hillsdale: Lawrence Erlbaum Associates; 1988.
8. Preuss HG, Bagchi D, Bagchi M, Rao CV, Dey DK, Satyanarayana S. Effects of a natural extract of (−)-hydroxycitric acid (HCA-SX) and a combination of HCA-SX plus niacin-bound chromium and *Gymnema sylvestre* extract on weight loss. *Diabetes, Obesity and Metabolism* 2004;**6**:171—80.
9. Vasques CA, Rossetto S, Halmenschlager G, Linden R, Heckler E, Fernandez MS, et al. Evaluation of the pharmacotherapeutic efficacy of *Garcinia cambogia* plus *Amorphophallus konjac* for the treatment of obesity. *Phytotherapy Research* 2008;**22**:1135—40.
10. Hackman RM, Havel PJ, Schwartz HJ, Rutledge JC, Watnik MR, Noceti EM, et al. Multinutrient supplement containing ephedra and caffeine causes weight loss and improves metabolic risk factors in obese women: a randomized controlled trial. *International Journal of Obesity (Lond)* 2006;**30**: 1545—56.
11. Kovacs EM, Westerterp-Plantenga MS, de Vries M, Brouns F, Saris WH. Effects of 2-week ingestion of (−)-hydroxycitrate and (−)-hydroxycitrate combined with medium-chain triglycerides on satiety and food intake. *Physiology and Behaviour* 2001;**74**:543—9.
12. Mattes RD, Bormann L. Effects of (−)-hydroxycitric acid on appetitive variables. *Physiology and Behaviour* 2000;**71**:87—94.
13. Maki KC, Reeves MS, Farmer M, Yasunaga K, Matsuo N, Katsuragi Y, et al. Green tea catechin consumption enhances exercise-induced abdominal fat loss in overweight and obese adults. *Journal of Nutrition* 2009;**139**:264—70.
14. Nagao T, Hase T, Tokimitsu I. A green tea extract high in catechins reduces body fat and cardiovascular risks in humans. *Obesity (Silver Spring)* 2007;**15**:1473—83.
15. Kovacs EM, Lejeune MP, Nijs I, Westerterp-Plantenga MS. Effects of green tea on weight maintenance after body-weight loss. *British Journal of Nutrition* 2004;**91**:431—7.
16. Kuriyan R, Raj T, Srinivas SK, Vaz M, Rajendran R, Kurpad AV. Effect of *Caralluma fimbriata* extract on appetite, food intake and anthropometry in adult Indian men and women. *Appetite* 2007;**48**:338—44.
17. Blom WA, Abrahamse SL, Bradford R, Duchateau GS, Theis W, Orsi A, et al. Effects of 15-d repeated consumption of Hoodia gordonii purified extract on safety, ad libitum energy intake, and body weight in healthy, overweight women: a randomized controlled trial. *American Journal of Clinical Nutrition* 2011;**94**:1171—81.
18. Ngondi JL, Etoundi BC, Nyangono CB, Mbofung CM, Oben JE. IGOB131, a novel seed extract of the West African plant Irvingia gabonensis, significantly reduces body weight and improves metabolic parameters in overweight humans in a randomized double-blind placebo controlled investigation. *Lipids in Health and Disease* 2009;**8**:7.
19. Udani J, Hardy M, Madsen DC. Blocking carbohydrate absorption and weight loss: a clinical trial using Phase 2 brand proprietary fractionated white bean extract. *Alternative Medicine Review* 2004;**9**:63—9.
20. Chen HL, Sheu WH, Tai TS, Liaw YP, Chen YC. Konjac supplement alleviated hypercholesterolemia and hyperglycemia in type 2 diabetic subjects — a randomized double-blind trial. *Journal of the American College of Nutrition* 2003;**22**:36—42.
21. Chevassus H, Gaillard JB, Farret A, Costa F, Gabillaud I, Mas E, et al. A fenugreek seed extract selectively reduces spontaneous fat intake in overweight subjects. *European Journal of Clinical Pharmacology* 2010;**66**:449—55.
22. Allison DB, Fontaine KR, Heshka S, Mentore JL, Heymsfield SB. Alternative treatments for weight loss: a critical review. *Critical Review of Food Science and Nutrition* 2001;**41**:1—28 [discussion 39—40].
23. Egger M, Smith GD. Bias in location and selection of studies. *British Medical Journal* 1998;**316**:61—6.
24. Easterbrook PJ, Berlin JA, Gopalan R, Matthews DR. Publication bias in clinical research. *Lancet* 1991;**337**:867—72.
25. Ernst E, Pittler MH. Alternative therapy bias. *Nature* 1997;**385**:480.
26. Pittler MH, Abbot NC, Harkness EF, Ernst E. Location bias in controlled clinical trials of complementary/alternative therapies. *Journal of Clinical Epidemiology* 2000;**53**:485—9.
27. Schulz K, Altman DG, Moher D, for the CONSORT Group. CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. *Annals of Internal Medicine* 2010;**152**(11):726—32.