Health Benefits of Cinnamon Supplement:

_in vitro_ and _in vivo_

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Abstract

Changing lifestyle and dietary habits, for example using herbal medicines, can influence the management and progression of some diseases, such as cardiovascular disease (CVD), diabetes and obesity. This thesis describes a series of in vitro experiments and human studies; the aims were to investigate the potential health benefits of Cinnamomum cassia (C. cassia) and Cinnamomum zeylanicum (C. zeylanicum) extracts in vitro, and C. cassia supplements on biomarkers of glucose, lipid profiles, weight, blood pressure, insulin and inflammatory markers.

Both extracts were found to be rich in polyphenols and proanthocyanidins which can act as effective free radical scavenging compounds in vitro. Both cinnamon types dose-dependently reduced the rapidly available glucose (RAG) and increased the slowly available glucose (SAG) values of cornflakes, possibly due to their polyphenolic compounds which have the capacity to inhibit carbohydrate digesting enzymes, particularly α-glucosidase and α-amylase. The preliminary randomised cross-over control human study investigated the effect of ingesting capsules of 1 g C. cassia prior to consuming cornflakes (a high starch food) on the glycaemic response in healthy participants. The results showed there were no significant differences in glucose response, nor in the incremental area under the curve for the cinnamon supplement or the placebo. In an 8-week randomised controlled human study, 5 g of C. cassia supplementation in overweight individuals reduced body weight (P=0.01), plasma non-esterified fatty acid levels (P=0.017), systolic (P=0.01) and diastolic blood pressure (P=0.02), without significantly affecting LDL, CHO and HDL levels as well as fasting insulin and glucose levels. There were no significant effects of cinnamon supplementation on cytokine and adhesion molecule levels. However, IL-6, IL-8, TNF-α, IL-1-α, MCP-1, sPSEL levels were significantly altered due to time in both cinnamon and control groups.

In conclusion, cinnamon supplementation (5 g/d for eight weeks) produced important health benefits in vitro and in vivo, therefore it may be useful as a natural herbal remedy for obesity and CVD.
Statement of originality

This thesis and the work to which it refers are the results of my own efforts. Any ideas, data, images or text resulting from the work of others (whether published or unpublished) are fully identified as such within the work and attributed to their originator in the text, bibliography or in footnotes. This thesis has not been submitted in whole or in part for any other academic degree or professional qualification. I agree that the University has the right to submit my work to the plagiarism detection service Turnitin in UK for originality checks. Whether or not drafts have been so-assessed, the University reserves the right to require an electronic version of the final document (as submitted) for assessment as above.
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AGEs</td>
<td>Advanced glycation end-products</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>C. cassia</td>
<td>Cinnamomum cassia</td>
</tr>
<tr>
<td>C. zeylanicum</td>
<td>Cinnamomum zeylanicum</td>
</tr>
<tr>
<td>CAMs</td>
<td>Cell adhesion molecules</td>
</tr>
<tr>
<td>CHO</td>
<td>Cholesterol</td>
</tr>
<tr>
<td>CIU</td>
<td>Clinical investigation unit</td>
</tr>
<tr>
<td>COX-2</td>
<td>Cyclooxygenase-2</td>
</tr>
<tr>
<td>CP</td>
<td>Cinnamon polyphenols</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>DMAC</td>
<td>4-Dimethylaminocinnamaldehyde</td>
</tr>
<tr>
<td>DP</td>
<td>Degree of polymerisation</td>
</tr>
<tr>
<td>DPPH</td>
<td>2,2-Diphenyl-1-1-Picrylhydrazyl</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic Acid</td>
</tr>
<tr>
<td>EGF</td>
<td>Endothelial growth factor</td>
</tr>
<tr>
<td>FPGT</td>
<td>Fasting plasma glucose test</td>
</tr>
<tr>
<td>GI</td>
<td>Glycaemic index</td>
</tr>
<tr>
<td>GOD</td>
<td>Glucose oxidase</td>
</tr>
<tr>
<td>HDL</td>
<td>High density lipoprotein</td>
</tr>
<tr>
<td>HOMA</td>
<td>Homeostasis model assessment</td>
</tr>
<tr>
<td>hs-CRP</td>
<td>High sensitive C-reactive protein</td>
</tr>
<tr>
<td>iAUC</td>
<td>Incremental Area under the Curve</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>Intercellular adhesion molecule-1</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Interferon-γ</td>
</tr>
<tr>
<td>Ig</td>
<td>Immunoglobulin</td>
</tr>
<tr>
<td>II</td>
<td>Insulin indices</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>-------------</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>LC-MS</td>
<td>Liquid Chromatography Mass Spectrometer</td>
</tr>
<tr>
<td>LDL</td>
<td>Low density lipoprotein</td>
</tr>
<tr>
<td>MCP-1</td>
<td>Monocyte chem attract protein-1</td>
</tr>
<tr>
<td>MetS</td>
<td>Metabolic syndrome</td>
</tr>
<tr>
<td>NAFLD</td>
<td>Non-alcoholic fatty liver disease</td>
</tr>
<tr>
<td>NEFA</td>
<td>None-estrified fatty acids</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>OGTT</td>
<td>Oral glucose tolerance test</td>
</tr>
<tr>
<td>PAC</td>
<td>Proanthocyanidin</td>
</tr>
<tr>
<td>POD</td>
<td>Peroxidase</td>
</tr>
<tr>
<td>pQCT</td>
<td>peripheral Quantitative Computer Tomography</td>
</tr>
<tr>
<td>PVPP</td>
<td>Poly-vinylpolypyrrolidone</td>
</tr>
<tr>
<td>RAG</td>
<td>Rapidly Available Glucose</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive Oxygen Species</td>
</tr>
<tr>
<td>SAG</td>
<td>Slowly Available Glucose</td>
</tr>
<tr>
<td>ESEL/ sESEL</td>
<td>E-selectin</td>
</tr>
<tr>
<td>LSEL/ sLSEL</td>
<td>L-selectin</td>
</tr>
<tr>
<td>PSEL/ sPSEL</td>
<td>P-selectin</td>
</tr>
<tr>
<td>TAG</td>
<td>Triacylglycerides</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumour necrosis factor alpha</td>
</tr>
<tr>
<td>Trab A</td>
<td>Trabecular Area</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>Vascular cell adhesion molecule-1</td>
</tr>
<tr>
<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
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CHAPTER 1
Chapter 1

Introduction

1.1 General introduction

The improvement of diet and lifestyle is a key strategy to help prevent chronic diseases such as cardiovascular disease (CVD), diabetes, cancer and obesity (Buttar et al., 2005). Changes in lifestyle could include: pursuing physical activity, reducing sugar intake, replacing refined grains with whole grains, eating plenty of fruit and vegetables, and limiting calorie intake (Lichtenstein et al., 2006). Fruits and vegetables are a good source of vitamins, fibre and phytochemicals; phytochemicals are bioactive non-nutrient compounds, such as polyphenols, alkaloids, tannins and flavonoids, which are found in fruits, vegetables, herbs and spices, which may provide health benefits as well as playing an important role in the prevention of chronic diseases such as atherosclerosis (Liu, 2003; Lichtenstein et al., 2006). In Asia, with assistance from the World Health Organisation (WHO), they are introducing and developing the use of herbs into primary health care (de Boer and Cotingting, 2014).

Herbs are defined as any part of the plant, including the leaves, stems, flowers, roots, barks and seeds, and herbal products may contain a single herb or a combination of several different herbs, which are believed to have complementary effects (Bent and Ko, 2004). Spices are dried herbs and are primarily used to enhance the flavour of foods (Dragland et al., 2003; Opara and Chohan, 2014). Herbs and spices have been identified as sources of various phytochemicals (Dragland et al., 2003), and when herbs and spices are used as a medicinal treatment, they are called medicinal herbs (Opara and Chohan, 2014). Herbal medicines can play an important role in the management and prevention of some disease such as diabetes, obesity, inflammation and CVD, and the use of herbal remedies in developed countries, such as the USA, has increased dramatically from 2.9 % in 1990, to 18 % in 2007 (Eisenberg et al., 2011). Moreover, a significant number of patients used herbal remedies for over a one-year period, for example 23 % in Denmark and 49 % in France. These data were compiled from public opinion surveys between 1985 and 1992 (Fisher and Word, 1994). In Saudi Arabia, 24 % of diabetic patients who were attending a primary care facility in Arar City used herbal medicines during the previous six months (Al-Ajaji et
al., 1998), and further studies in Saudi Arabia showed that 17.4 % of diabetic patients who were attending the outpatient clinics in hospitals used some form of herbs, indicating how wide spread herbal use is (Al-Rowais, 2002).

1.2 History of herbal medicine use

The use of herbal medicines has a long history, more than 50 % of clinical drugs come from herbal products or their derivatives (Kadir et al., 2012). For example, metformin, which is a drug that lowers blood glucose by increasing the body’s cell sensitivity to insulin in type 2 diabetes, is derived from French lilac, and was a herbal remedy used to treat diabetes in the Middle Ages (Ryan et al., 2001). Similarly, ginger was used to treat sore throats, which is still used currently (Jungbauer and Medjakovic, 2012).

The use of herbal supplements has also been proved effective, for example in a double blind crossover study it was shown that consuming 1200 mg/day of curcumin as a supplement, plus the patient’s medication (phenyibutazone 300 mg/day), for two weeks improved morning stiffness, walking time, and joint swelling in people with rheumatoid arthritis, while there was no changed in control (Deodhar et al., 1980). Moreover, herbs and spices such as cinnamon, cloves, tea, oregano, and nutmeg, have been shown to possess insulin-like biological activity (Broadhurst et al., 2000).

Cinnamon has been used since ancient times and is the inner bark of the Cinnamomum tree, which belongs to Lauraceae family. The main types of cinnamon are C. zeylanicum (also known as C. verum, C. cylone) which is produced in Sri Lanka, and C. cassia which is mainly grown in China. The term ‘Cinnamomum’ is derived from Greek and means ‘sweet wood’ (Ravindran et al., 2004; Hamidpour et al., 2015).

Current research, both in vitro and in animals in vivo, suggests that cinnamon has anti-inflammatory, anti-microbial, antibacterial, anti-oxidant, anti-diabetic, anti-obesity and immunomodulatory effects (Anderson et al., 2004; Wang et al., 2009; Dudonné et al., 2009; Lee et al., 2005).

1.2.1 The major bioactive components of cinnamon

Cinnamon has been shown to consist of phenolic compounds, tannins, sugars, mucilage, resins and volatile oils, all of which have different properties. The main volatile oil is cinnamaldehyde which contributes 80-90 % of the volatile oil content of Cinnamomum cassia, and 60-80 % of the volatile oil of Cinnamomum zeylanicum
The composition of cinnamon bark oil has been further investigated by Kim et al., (2015) who identified the main components using GC-MS analysis and they found 17 different components (Table 1.1). As with the previous studies, cinnamaldehyde was the predominant component and accounted for 64.49% of cinnamon bark oil. In addition, eugenol (16.57%) and linalool (4.82%) were also important constituents of cinnamon bark oil, which suggests these are also likely to cause the medicinal properties seen in previous studies.

**Table 1.1** GC-MS analysis of cinnamon bark oil composition (source Kim et al., 2015).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Composition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Pinene</td>
<td>3.02</td>
</tr>
<tr>
<td>β-Pinene</td>
<td>1.23</td>
</tr>
<tr>
<td>ρ-Cymene</td>
<td>0.81</td>
</tr>
<tr>
<td>Limonene</td>
<td>2.53</td>
</tr>
<tr>
<td>Linalool</td>
<td>4.82</td>
</tr>
<tr>
<td>α-Terpineol</td>
<td>0.26</td>
</tr>
<tr>
<td>Benzaldehyde</td>
<td>1.80</td>
</tr>
<tr>
<td>Cinnamaldehyde</td>
<td>64.49</td>
</tr>
<tr>
<td>Safrole</td>
<td>0.73</td>
</tr>
<tr>
<td>Eugenol</td>
<td>16.57</td>
</tr>
<tr>
<td>α-Copaene</td>
<td>0.12</td>
</tr>
<tr>
<td>trans-Caryophyllene</td>
<td>1.10</td>
</tr>
<tr>
<td>Cinnamyl acetate</td>
<td>1.15</td>
</tr>
<tr>
<td>α-Humulene</td>
<td>0.20</td>
</tr>
<tr>
<td>Acetyl eugenol</td>
<td>0.48</td>
</tr>
<tr>
<td>O-mentha-1(7),8-dien-3-ol</td>
<td>0.20</td>
</tr>
<tr>
<td>Benzylbenzoate</td>
<td>0.49</td>
</tr>
</tbody>
</table>

The composition of cinnamon has also been investigated by other groups who found that cinnamon bark contained high levels of condensed tannins which were comprised of 23.2% proanthocyanidins, and 3.6% epi-catechins (Shan et al., 2007). All of which may be involved in producing the medicinal effects previously observed.

The current *in vitro* (Table 1.2) and *in vivo* studies involving cinnamon suggest that it could be used to treat and prevent chronic diseases, such as obesity and diabetes.
mellitus, which have a high prevalence in the developed world (Ranasinghe et al., 2013) but the actual mechanism of action needs to be determined in humans.

Table 1.2 The benefit effects of cinnamon from *in vitro* studies.

<table>
<thead>
<tr>
<th>References</th>
<th>Cinnamon impact</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jarvill-Taylor et al., 2001</td>
<td>Cinnamon activated insulin receptors and enhance glucose uptake in 3T3-L1 pre-adipocytes</td>
</tr>
<tr>
<td>Peng et al., 2008</td>
<td>Cinnamon inhibited advance glycation end products (AGEs) in bovine serum albumin glucose model</td>
</tr>
<tr>
<td>Hong et al., 2002</td>
<td>Cinnamon inhibited cyclooxygenase (COX-2) activity in mouse macrophage RAW 264.7 cells</td>
</tr>
<tr>
<td>Lu et al., 2011</td>
<td>Cinnamon significantly increased the consumption of extracellular glucose in insulin-resistant HepG2 cells</td>
</tr>
<tr>
<td>Friedman et al., 2002</td>
<td>Cinnamon had a potent anti-microbial activity against <em>Escherichia coli</em> (<em>E. coli</em>)</td>
</tr>
<tr>
<td>Mastura et al., 1999</td>
<td>Cinnamon had anti-fungal properties against <em>Candida albicans</em></td>
</tr>
<tr>
<td>Premanathan et al., 2000</td>
<td>Cinnamon inhibited the virus-induced cytopathogenicity in MT-4 cells infected with HIV</td>
</tr>
</tbody>
</table>

1.3 Overweight and obesity

One of the most prevalent health conditions in the world is obesity, which is associated with various metabolic abnormalities including insulin resistance and dyslipidaemia (Klop et al., 2013). Furthermore, obesity can increase the risk of developing diabetes, hypertension and cardiovascular disease (CVD), therefore, there is a great deal of interest in preventing and treating this condition (Klop et al., 2013).

1.3.1 Definition and prevalence

Obesity is defined as the excess accumulation of body fat (Hawkesworth, 2013), and the most common method used to classify whether an individual is overweight or obese is based on their Body Mass Index (BMI). This is calculated by dividing the body weight
of an individual in kg by their height in meters squared (kg/m\(^2\)). According to the World Health Organisation (WHO, 2004) individuals with a BMI of 25-29.9 kg/m\(^2\) are classified as overweight, whereas individuals with a BMI of over 30 kg/m\(^2\) are classified as obese.

Research shows that obesity increases the risk of developing type 2 diabetes, dyslipidaemia, hypertension and CVD (Mokdad et al., 2003; Parikh et al., 2015). The prevalence of individuals who are overweight exceeds 37 % of the population in most developing countries (Tully et al., 2015), and globally, more than 1.4 billion adults were overweight in 2008 (WHO, 2013). This is further exemplified by the fact that in the USA there were 154.7 million overweight or obese individuals recorded in 2011 (American Heart Association, 2013), and in the UK, the prevalence of obesity in adults rose from 15 % in 1993 to 25 % in 2013 (National Obesity Observatory, 2013). A recent study in Saudi Arabia confirmed that the prevalence of obesity was 28.7 %, which was determined from a large household survey in 2013 (Memish et al., 2014). The results of a recent systematic study of the global prevalence of adults who are overweight or obese in various countries between 1980 and 2013 (Table 1.3) shows the prevalence of this condition is worldwide (Ng et al., 2014).
Table 1.3 National estimates of the prevalence of individuals who are overweight or obese in 2013*

<table>
<thead>
<tr>
<th>Country</th>
<th>Overweight and obese</th>
<th>Overweight and obese</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men % (95 % uncertainty intervals, UIs)</td>
<td>Women % (95 % uncertainty intervals, UIs)</td>
</tr>
<tr>
<td>New Zealand</td>
<td>71·4 (69·6–73·3)</td>
<td>60·0 (57·8–62·2)</td>
</tr>
<tr>
<td>Mexico</td>
<td>66·8 (64·9–68·6)</td>
<td>71·4 (69·5–73·2)</td>
</tr>
<tr>
<td>Russia</td>
<td>54·3 (51·5–57·1)</td>
<td>58·9 (56·3–61·4)</td>
</tr>
<tr>
<td>USA</td>
<td>70·9 (69·2–72·5)</td>
<td>61·9 (59·8–63·8)</td>
</tr>
<tr>
<td>Bahrain</td>
<td>67·7 (65·3–70·2)</td>
<td>75·2 (72·8–77·5)</td>
</tr>
<tr>
<td>Egypt</td>
<td>71·2 (68·9–73·7)</td>
<td>75·2 (72·8–77·5)</td>
</tr>
<tr>
<td>Kuwait</td>
<td>74·5 (72·4–76·6)</td>
<td>84·3 (82·6–86·1)</td>
</tr>
<tr>
<td>Libya</td>
<td>70·6 (68·1–73·1)</td>
<td>77·0 (74·6–79·3)</td>
</tr>
<tr>
<td>Qatar</td>
<td>75·7 (73·8–77·4)</td>
<td>78·5 (77·0–80·1)</td>
</tr>
<tr>
<td>Saudi Arabia</td>
<td>69·0 (67·1–70·7)</td>
<td>74·2 (72·3–76·0)</td>
</tr>
<tr>
<td>United Arab Emirates</td>
<td>66·1 (63·6–68·8)</td>
<td>60·6 (57·4–63·4)</td>
</tr>
<tr>
<td>Germany</td>
<td>64·3 (61·9–66·8)</td>
<td>49·0 (46·5–51·4)</td>
</tr>
<tr>
<td>Ireland</td>
<td>66·4 (63·9–68·8)</td>
<td>50·9 (48·3–53·6)</td>
</tr>
<tr>
<td>UK</td>
<td>66·6 (65·3–68·0)</td>
<td>57·2 (55·7–58·6)</td>
</tr>
</tbody>
</table>

*The participants in this study were adults over the age of 20 who were located in 188 countries and 21 GBD regions, source (Ng et al., 2014).

As a result of the increasing rate of obesity, there has been a significant increase in interest in using of complementary and herbal medicines for treating obesity, together with suitable diet and exercise programs for losing and maintaining weight (Westerterp-Plantenga et al., 2006; Auvichayapat et al., 2008).

1.3.2 Abdominal obesity

Waist circumference provides an indication of the distribution of body fat, and compared to BMI, it is a better predictor of the health risks associated with an excess of weight, whereas BMI can be used as an indicator of overall body fat content (Zhu et al., 2002; Janssen et al., 2004).
There are studies that show abdominal obesity is linked with hyperinsulinaemia, insulin resistance, diabetic dyslipidaemia, hypertension, CVD, and inflammation (Sowers, 2003; Aoki et al., 2015). An overall reduction in weight has health benefits such as reducing blood pressure and inflammatory markers, and improving lipid concentrations (Klein et al., 2004), and in addition, weight loss, and more specifically loss of abdominal obesity, can improve insulin sensitivity (Norris et al., 2005).

1.3.3 Health consequences of obesity

There is strong evidence for the association between obesity and other diseases, for example, obesity increases the risk of developing type 2 diabetes 12-fold compared with individuals with BMI within the normal range (Kumanyika et al., 2008). Also, obesity increases the risk of developing CVD by 20 %, type 2 diabetes by more than 30 % (McQuigg et al., 2008), and central (abdominal) obesity contributes to the development of insulin resistance (Rader, 2007).

Insulin resistance has been shown to increase the development of hypertension and CVD (Grundy, 1998; McFarlane et al., 2001). Insulin resistance is considered as a pro-inflammation state via elevated inflammatory marker levels (Meigs et al., 1997; McFarlane et al., 2001). Obesity, type 2 diabetes mellitus, and metabolic syndrome have increased dramatically in the past decades (Mokdad et al., 2001). The increasing health effects related to obesity result in higher costs to the world’s health care systems (Huang et al., 2009), and there are also implications for the workforce and reducing absenteeism through ill-health. Therefore, it is important that diet and lifestyle interventions are identified to reduce obesity and their associated diseases.

1.3.3.1 Insulin resistance

Insulin resistance (IR) is a biological reduction in the response to insulin; it is associated with metabolic syndrome, which includes central obesity, low levels of high-density lipoprotein (HDL) cholesterol, high triglyceride levels, high low-density lipoprotein (LDL) cholesterol levels, glucose intolerance, type 2 diabetes mellitus, and hypertension (Greenfield and Campbell, 2004). Currently, there is no single method that can be used directly to determine IR, however the homeostasis model assessment [HOMA] index is widely used as a validated way to determine IR [HOMA-IR] index and insulin sensitivity, and is based on the values of fasting blood glucose and insulin (Romualdo et al., 2014).
1.3.3.1.1 The role of weight loss in improving IR

One of the most important complications related to obesity is IR, which is correlated to the pathogenesis of chronic diseases, such as type 2 diabetes and CVD (Facchini et al., 2001). Schenk et al. (2009) have reported that insulin sensitivity was improved by weight loss, either by diet-only or a diet and exercise program in obese women. This study randomly divided obese women into lifestyle intervention groups of either weight loss without exercise (WL) (n = 7), or a weight loss + exercise program (WL+EX) (n = 10). Insulin sensitivity (IS) and rates of systemic fatty acid appearance and disappearance were measured at the baseline, and after losing 12 % of their body weight. They concluded that losing weight reduced fatty acid mobilization and uptake and improved insulin sensitivity. Overall these studies suggest that weight loss is very important for IS and obesity.

1.3.3.2 Metabolic syndrome

Metabolic syndrome (MetS) is a metabolic abnormality associated with an increased risk of developing CVD and type 2 diabetes (Figure 1.1). Indicators of MetS include abdominal obesity with a waist circumference >102 cm for men and >88 cm for women, hypertension (systolic blood pressure >130 mm Hg or diastolic blood pressure >85 mm Hg), dyslipidaemia, IR, a pro-inflammatory state, and hyperglycaemia (Kaur, 2014). The prevalence of MetS was reported to be approximately 17.8–34.0 % of the population in Europe and 12.8–41.1 % in Asia, and it has been shown to be increasing over time, suggesting this is a large and increasing problem (Athyros et al., 2005; Grundy, 2008). IR and MetS have been shown to be associated with disease progression in non-alcoholic fatty liver disease (NAFLD) (Pais et al., 2015), and also with increased non-esterified fatty acid (NEFA) levels (Maison et al., 2000). A systematic study has shown that NAFLD and fatty livers have increased in the general population, which in turn can lead to chronic liver disease (Argo et al., 2009). NAFLD is present in 70-80 % of diabetic and obese patients, therefore efforts to reduce obesity should also help to reduce the incidence of NAFLD (Soffientini et al., 2014).
Figure 1.1: Representation of the correlation between obesity and other aspects of metabolic syndrome (adapted from the following sources: Taube et al., 2009; Hebbard and George, 2011; Jungbauer and Medjakovic, 2012). Excessive energy intake over expenditure leads to an enlargement of adipose tissue. These tissues release pro-inflammatory cytokines such as IL-6 and TNF-α, as well as NEFA. Hypertrophic adipocytes are infiltrated by macrophages and activated by monocytes. These cause a chronic inflammation, leading to metabolic syndrome, insulin resistance, type 2 diabetes, and arteriosclerosis.

1.3.3.3 The effect of inflammatory status

Inflammation is a reactive response of the immune system to infection and tissue damage; it is a protective action which increases the movement of defence cells from the bloodstream into an infected area (Pomin, 2015). Acute inflammatory responses generally progress over several days, whereas chronic inflammatory responses
progress over a much longer time period, from months to years, to a lifetime, such as in atherosclerosis, obesity, and autoimmune diseases (Nayak et al., 2013; Fleit, 2014). During an inflammatory response neutrophil cells are activated, these release pro-inflammatory cytokines such as interleukins (IL-1, IL-6, IL-12 and IL-18) and tumour necrosis factor (TNF-α) (Jungbauer and Medjakovic, 2012). This leads to the activation of nitric oxide synthase (NOS) and cyclooxygenase-2 (COX-2). The activated neutrophils travel into the tissues and recruit macrophages, which release factors to heal the effected site and clear any pathogens (Jungbauer and Medjakovic, 2012). Continual low-grade inflammation and persistent infections cause chronic inflammation, which can be detected by increased levels of inflammatory markers such as C-reactive protein (CRP) and TNF-α (Libby et al., 2002). Immune problems can be further exacerbated by a disorder in the immune system, which leads to an accumulation of leukocytes that induce DNA damage in proliferating cells through their generation of reactive oxygen (ROS) and nitrogen species that are normally produced by these cells to fight infection (Coussens and Werb, 2002).

Increasing adiposity in obesity is associated with recruitment of inflammatory cells, such as macrophages and monocytes, into the adipose tissue. These cells can release cytokines, such as IL-6, IL-1β, and TNF-α, that are capable of impeding insulin signalling and therefore their action is related to the development of IR (Figure 1.1) (Fain, 2010; Deer et al., 2015). Obesity, diabetes and CVD are associated with elevated markers of chronic inflammation, such as CRP, IL-6 and TNF-α (Berg and Scherer, 2005), which suggests the inflammatory state might be responsible for IR and the other disorders associated with obesity, such as hyperlipidaemia and MetS (Calabrò et al., 2010). By reducing the extent of obesity, the levels of these markers should also be reduced, which could have beneficial effects on patient health.

### 1.3.3.4 Dyslipidaemia

Lipoproteins are a complex of lipid and protein that are synthesised in the liver and the intestines, and they are responsible for transporting lipids in the blood (Jain et al., 2007). There are four classes of lipoproteins: low density lipoproteins (LDL), high density lipoproteins (HDL), very low density lipoproteins (VLDL), and triglycerides (TAG) (Jain et al., 2007).
Dyslipidaemia is a common complication of obesity and consists of an increase in both fasting and postprandial TAG levels, an increase in LDL, VLDL, NEFA (for adipose tissue), and low HDL concentration in the body. (Klop et al., 2013; Soffientini et al., 2014). Dyslipidaemia is a major risk factor in the development of coronary heart disease and has become one of the most common public health problems as it of associated with IR, MetS, obesity and diabetes (Garvey et al., 2003). A review of the area has shown that a reduction of 10 % of the weight in healthy men by diet alone, can decrease the non-fasting TAG by between 26 - 46 % which suggests a reduction in dyslipidaemia (Klop et al., 2013). However, a meta-analysis of anti-obesity drugs reported that weight was significantly reduced by 3.13 kg (95 % CI: 24.00 to 22.26) compared with placebo but the treatment did not improve TAG, LDL and CHO levels (Zhou et al., 2012). Overall this suggests that more research is needed to determine the effect of weight loss on dyslipidaemia.

1.4 Diabetes mellitus

1.4.1 Definition and classification

Diabetes mellitus (DM) is a serious metabolic disorder characterised by chronic hyperglycaemia and additional disturbances of carbohydrate, fat and protein metabolism. (WHO, 1999; American Diabetes Association (ADA), 2015. It results from either too little production of the hormone insulin, which is released by β-cells located in the pancreas, or the failure of insulin to function appropriately (WHO, 1999; American Diabetes Association (ADA), 2015).

The number of people with diabetes in the world is expected to reach 366 million by 2030, with increases worldwide, for example, in the Middle Eastern Crescent region the estimated number of people with DM was 20.05 million in 2000 and this is expected to rise to 52.79 million by 2030 (Wild et al., 2004). In Saudi Arabia, the prevalence of DM in adults was 23.7 % between 1995 and 2000 (Al-Nozha et al., 2004), which has been shown to have risen significantly by a recent study by Al-Rubeaan et al. (2014) which highlighted that more than 50 % of the Saudi population was either diabetic or pre-diabetic between 2007 and 2009.

This increase in diabetes is thought to be due to population growth and urbanization, but also due to an ageing population and the increased prevalence of obesity combined with reduced physical activity (Wild et al., 2004). According to ADA (2015), DM is
classified into four clinical groups; the first group is type 1 diabetes, which is also known as Insulin-Dependent Diabetes Mellitus (IDDM). Type 1 diabetes can occur at any age, but it is most often diagnosed in children, adolescents or young adults. This type of diabetes results from β-cell destruction, usually leading to absolute insulin deficiency. The second group is type 2 diabetes, which is also called Non-Insulin-Dependent Diabetes Mellitus (NIDDM). Type 2 is the most common form of diabetes and usually occurs progressively among the adult population. Type 2 diabetes results from a progressive insulin secretory defect and leads to IR.

Other types of diabetes exist due to other causes, such as genetic defects in β-cell function or insulin action, diseases of the exocrine pancreas (such as pancreatic fibrosis), and drug- or chemical-induced diabetes (such as in the treatment of AIDS or after organ transplantation). The final class is gestational diabetes mellitus (GDM) which is diagnosed during pregnancy.

IR is classified as a reduced sensitivity of a target cell or a whole organism to insulin, and is also linked to hyperinsulinaemia (Shanik et al., 2008). IR has been assigned a key role in the metabolic disturbances associated with obesity and type 2 diabetes (Shanik et al., 2008). Furthermore, IR in the brain is suggested to be a factor that relates to Alzheimer’s disease among type 2 diabetic patients, which could be considered as a new type of diabetes (de la Monte et al., 2008).

1.4.2 Diagnosis of DM

The common symptoms of diabetes include fatigue, increased appetite, excessive thirst, increased urination and increased frequency of infections. The ADA (2015) suggests that three blood tests are required for an accurate diagnosis of diabetes, and a fasting plasma glucose test (FPGT) is the easiest to use. A fasting level of blood glucose of 100 to 125 mg/dl is considered pre-diabetic, and one higher than 126 mg/dl (＞7.0 mmol/l) on two occasions indicates the patient has diabetes. An oral glucose tolerance test (OGTT) is the second procedure that can be used to detect diabetes; this also involves a blood test and is more sensitive and specific than the FPGT to diagnose diabetes. The OGTT measures the blood glucose level after a fast and then two hours after drinking a glucose rich drink containing 75 g of anhydrous glucose dissolved in 250 ml of water. A glucose reading of more than 200 mg/dl (＞11.1 mmol/l) indicates the presence of diabetes in the individual. Haemoglobin A1c (HbA1c) testing
is the third test used to diagnose diabetes; in this case a level higher than 6.4 % of haemoglobin A1c confirms a diabetes diagnosis.

There are many factors that may increase the prevalence of DM in the population such as age, gender, and lifestyle (Wild et al., 2004). The patterns of eating in some countries have changed dramatically in recent years, in particular in the quantity and quality of dietary carbohydrates. As such it is useful to consider sources of dietary carbohydrates and the way in which they can be defined.

1.5 Dietary carbohydrates

1.5.1 Definition

Carbohydrates are the most abundant organic components in most fruits, vegetables and cereal grains, and they are constructed from the atoms of carbon, oxygen and hydrogen (Garrett and Grisham, 2012). Carbohydrates are carbon-based molecules with an empirical formula for of \((\text{CH}_2\text{O})_n\), where \(n = 3\) or more, and they are rich in hydroxyl groups. Carbohydrates are comprised of two major classes; the simple carbohydrate class includes monosaccharides and disaccharides, the latter of which consists of two-monosaccharide units joined by a covalent bond. The complex carbohydrate class includes oligosaccharides, which contain short chains of monosaccharides between 3-10 units joined by covalent bonds, and the polysaccharides which contain a long chain of monosaccharides of more than 10 units (Gropper et al., 2009; Garrett and Grisham, 2012; Berg et al., 2012).

1.5.2 Chemical structures

1.5.2.1 Monosaccharides

Monosaccharides consist typically of three to seven carbon atoms and are described either as aldoses (when they contain an aldehyde group) or ketoses (when they contain a keto group) (Gropper et al., 2009). They are classified by the number of carbon atoms in their structures (trioses, tetroses, pentoses, hexoses and heptoses), by their stereochemistry (D or L), and by the degree to which they are polymerized (disaccharides, oligosaccharides and polysaccharides) (Stipanuk, 2000; Berg et al., 2012). In the Fisher projection, when the hydroxyl group is on the right of C4, these sugars have the D-sugar designation, whereas L-sugars are the mirror images of D-sugars (Stipanuk, 2000).
The common monosaccharides are hexoses, such as glucose and fructose, which come in two isomeric forms; the D configuration is much more important nutritionally than the L configuration as the D isomers exist more frequently in nature and they are key in carbohydrate digestion and their subsequent metabolism (Figure 1.2).

**Figure 1.2:** The ring forms of L-glucose, D-glucose and fructose. (Source Berg et al., 2012).

### 1.5.2.2 Disaccharides

The simplest oligosaccharides are the disaccharides, which consist of two monosaccharide units linked by an acetal bond, which is also called a glycosidic bond (Gropper et al., 2009; Garrett and Grisham, 2012). Glycosidic bonds are formed between a hydroxyl group of one monosaccharide unit and a hydroxyl group of the next unit in the polymer chain (Gropper et al., 2009; Garrett and Grisham, 2012). The glycosidic bond can be in an α or β in orientation depending on the form of the anomeric hydroxyl group, which can be either α or β, before the glycosidic bond was formed. For instance, the glycosidic bond may be designated α 1-4, β 1-4, or α 1-6 (Gropper et al., 2009). The most common disaccharides, which are the largest energy providing nutrient in the diet, are maltose, lactose and sucrose; maltose consists of two glucose units linked by an α 1-4 glycosidic bond, lactose is the main carbohydrate in milk and consists of D-galactose and D-glucose linked through a β 1-4 glycosidic bond, and sucrose is the most common disaccharide used as a sweetener, and is commonly known as table sugar, and is composed of glucose and fructose (Gropper et al., 2008; Berg et al., 2012; Garrett and Grisham, 2012).
1.5.2.3 Polysaccharides

Polysaccharides are formed from high molecular weight polymers. There are two kinds of polysaccharides, homopolysaccharides which contain only one type of monosaccharide, and heteropolysaccharides which contain different types of monosaccharide. Homopolysaccharides are more important than heteropolysaccharides nutritionally due to their abundance in many natural foods (Gropper et al., 2009; Garrett and Grisham, 2012). Starch is the most common storage polysaccharide in plants such as cereal grains, potatoes and legumes, which are the most common carbohydrate containing foods consumed in the human diet (Englyst et al., 1996; Cummings et al., 1997; Gropper et al., 2008; Garrett and Grisham, 2012). In addition, carbohydrates, either as starch or as simple sugars, account for 40-50 % of the calories consumed in the Western diet, which shows their overall importance (Caspary, 1992). Starch can exist in two forms: amylase and amylopectin; amylase is a straight-chain polymer of D-glucose linked through α 1-4 glycosidic bonds, whereas amylopectin is a branched chain polymer linked via α 1-6 and α 1-4 glycosidic bonds (Figure 1.3). Amylopectin has a larger molecular weight compared to amylase, and therefore has a larger surface area per molecule, this results in amylopectin being more accessible to enzymatic digestion as it is easier to hydrolyze (Thorn et al., 1983). Amylose typically contributes approximately 15-20 % of the total starch content of foods and amylopectin 80-85 % (Cummings and Englyst, 1995; Englyst and Hudson, 1996; Cummings et al., 1997; Whitcomb and Lowe, 2007; Gropper et al., 2008; Garrett and Grisham, 2012). Barley and rice are examples of starch rich foods that are high in amylase (Zhou and Kaplan, 1997, Hu et al., 2004). The amylase: amylopectin ratio is a factor affecting starch digestibility and its physiological response, high amounts of amylase in starch products are sources of resistant starch (RS) that digests slowly, which might have potential health benefits. For example, they could slow the rise of postprandial blood glucose levels, and sustain blood glucose levels over time. Slow digestible starch foods produce a low glycaemic Index (GI) (Lehmann and Robin, 2007).
1.5.3 The digestion of carbohydrates

Polysaccharides and disaccharides are the most important dietary carbohydrates, and starch is the most common digestible polysaccharide in the human diet (Gropper et al., 2009). Both polysaccharides and disaccharides are hydrolysed by intestinal enzymes to their constituent monosaccharide units; this is the only form of carbohydrate that can be absorbed by the intestinal epithelium (Gropper et al., 2009). The digestion of polysaccharide starts in the mouth; the salivary glands release salivary α-amylase, which hydrolyses the α 1-4 glycosidic bonds in amylose and amylopectin, whereas the α 1-6 glycosidic bonds in amylopectin are resistant to α-amylase (Whitcomb and Lowe, 2007; Gropper et al., 2009). The enzymatic activity of salivary α-amylase is destroyed by the acidity of gastric juices (pH 2.0) in the stomach, consequently, there is no enzymatic digestion of starch in the stomach and the dextrins (low-molecular-weight carbohydrates produced by the hydrolysis of starch or glycogen) pass through without any further change into the small intestine (Gropper et al., 2009). The pancreas releases pancreatic α-amylase into the small intestine which hydrolyses α 1-4 glycosidic bonds in both amylose and amylopectin, and breaks down the dextrins into maltose (Gropper et al., 2009; Garrett and Grisham, 2012). On the brush border of the small intestine maltose is hydrolysed by maltase to produce glucose, and the α 1-4
glycosidic bonds in limit dextrins are hydrolysed by α-dextrinase to produce glucose (Gropper et al., 2009).

Some disaccharides and oligosaccharides are not digested in the mouth or stomach, such as lactose, sucrose, maltose and isomaltose; they can be digested in the microvilli of the intestinal mucosal cells of the upper small intestine (Gropper et al., 2009). Lactose is broken down by lactase into galactose and glucose, sucrose is hydrolysed by sucrase into glucose and fructose, and finally, isomaltose, which is the remaining disaccharide from the incomplete breakdown of amylopectin, is separated by isomaltase (α-dextrinase) to yield two molecules of glucose (Gropper et al., 2009).

1.5.4 The absorption of carbohydrates

Carbohydrates in the diet occur as monosaccharaides, disaccharides, oligosaccharides and polysaccharides, however, in the human intestinal tract, only the monosaccharides can be absorbed (Wright et al., 2003). Approximately 70-85% of the carbohydrates in the diet are absorbed as glucose, with the remainder being a mixture of fructose and galactose (Dikeman et al., 2004). The absorption of most of these monosaccharides occurs through the luminal surface of microvilli cells (the brush border); glucose and galactose absorption depends on the activity of the sodium glucose transporter 1 (SGLT1), which is a protein complex dependent on Na⁺, K⁺, and ATP as energy (Kellett et al., 2008). Glucose enters the absorptive cell by GLUT4 and SGLT1 in the brush border membrane and exits into the blood by Glucose Transporter 2 (GLUT2), which is a member of the facilitative glucose transporter family (Hediger and Rhoads, 1994; Kellett et al., 2008). However, fructose absorption is dependent on GLUT5, which is specific for fructose, it enters the cell by GLUT5 and exits the cell by GLUT2, which is the same member of the transporter family that moves glucose out of the cell (Kellett and Helliwell, 2000; Kellett, 2001).

The hormone involved in glucose absorption regulation is insulin, it is released by the β-cells of the pancreas when the blood glucose levels are elevated (Wright et al., 2003; Baumgard et al., 2016). Insulin plays many roles in glucose absorption; it increases the activity of enzymes that catalyze the synthesis of glycogen in the liver to produce glucose, and it stimulates the glucose transport protein GLUT4 in skeletal muscle and adipose tissue (Baumgard et al., 2016). Skeletal muscle both stores glucose as glycogen and oxidizes it to produce energy (Gropper et al., 2009). Studies have shown
that GLUT4 plays an essential role in regulating whole body glucose homeostasis, and is therefore vital for the correct functioning of glucose uptake and metabolism (Huang and Czech, 2007; Baumgard et al., 2016).

As highlighted above, carbohydrate is the main source of energy in the human diet. There are many methods to measure the quantity of the carbohydrates in food, as well as determining the release of glucose from starch-rich foods which will allow the amount of energy released to be determined. One of the most common methods for determining the speed of release of glucose from starchy foods is by using specific enzymes that break down the starch in vitro.

1.5.5 The measurement of carbohydrate availability in vitro

There are many different methods available that can be used to determine the total carbohydrate in foods, there are also many methods that use enzymes to determine the speed of glucose released from carbohydrate foods in vitro, for example enzyme based hydrolysis of starch (Englyst and Englyst, 2005). This approach uses pancreatic and brush border membrane enzymes to determine and measure the glucose released from a starch-rich food. The amount of glucose released is measured at specific times during hydrolysis to provide an indication of carbohydrate availability and speed of release (Englyst and Englyst, 2005). The two useful measurements obtained are the rapidly available glucose (RAG) and the slowly available glucose (SAG); these terms provide values for glucose that is released from starch, and other glucose yielding carbohydrates, that are available for absorption in the small intestine (Englyst and Englyst, 2005). These measurements can be used as a guide for the GI in vivo and provide a clear indicator of the effect of a starchy food on blood glucose levels (Englyst et al., 1999; Englyst et al., 2000; Englyst et al., 2003). Indeed, a significant correlation ($P < 0.001$) has been shown between the release of RAG, SAG, and GI (Englyst et al., 1992; Englyst et al., 1999). This shows that a food or beverage with a high RAG value would probably have a high GI, while a food or beverage with a low RAG value would have a low GI. It is important to know the GI of a food to allow an accurate measurement of energy intake to create a balanced diet.

1.6 Glycaemic index

Carbohydrates can be classified according to their physiological effect in the human body, and not just by their chemical structure (section 1.5.2); they can be classified by
their ease of digestion and absorption, and their effect on the elevation of blood glucose levels (Asp, 1996; Englyst et al., 2007). The GI is a method that was introduced by Jenkins and colleagues in 1981 to classify carbohydrate containing foods according to their impact on the postprandial glycaemic response (Jenkins et al., 1981). GI is defined as the incremental area under the two-hour blood glucose response curve, after consumption of a test food containing 50 g or 25 g of available carbohydrates, expressed as the percentage of the response to the same amount of carbohydrates from a standard food (e.g. white bread or glucose) taken by the same participants (Wolever, 1990; Wolever et al., 1991; FAO/WHO, 1997). The GI has been reported to be a more useful nutritional concept than the chemical classification of carbohydrates, especially as low GI foods are slowly digested and absorbed, and produce a gradual rise in the blood glucose level (Wolever, 1990; Jenkins et al., 2002). Therefore, low GI foods are generally considered to be favourable in the dietary management of type 2 diabetes, and are also considered important in reducing CVD and obesity (Granfeldt et al., 2006).

### 1.6.1 Measuring the GI of starch rich foods

According to the FAO/WHO guidelines (1997) the sample size required for a GI study is between 10 and 12 participants. Participants must refrain from consuming alcohol and caffeine-containing drinks for at least 24 hours prior to the test day. To measure fasting glucose and changes in glucose levels, capillary finger prick blood samples must be taken from the participants in the fasted state and then every 15 minutes during the first hour, and every 30 minutes throughout the second hour after the start of consumption of the test food or reference food, in random order, as this allows the changes over time to be observed. The amount of available carbohydrate in the test food, or reference food, should be 25 g or 50 g. The reference food, which is often a glucose drink, should be given to the participants three times on separate occasions to reduce the variability between experiments. Blood glucose is measured with an automatic analyser and plotted on a graph against the blood sampling time, as this will allow the incremental area under the curve (IAUC) of each blood glucose response to be calculated geometrically (Figure 1.4). The GI value is calculated as: (IAUC sample/IAUC standard) x 100 (Figure 1.5) (Wolever et al., 1991; Brouns et al., 2005).
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Figure 1.4: The incremental area under the curve (IAUC) equals the sum of the area A, B, C, D and F (Wolever et al., 1991; Brouns et al., 2005). The area under the curve can be calculated from the following equation:

\[
\text{Area} = \frac{At}{2} + At + \frac{(B-t)}{2} + Bt + \frac{(C-B)t}{2} + Ct + \frac{(D-C)t}{2} + Dt + \frac{(E-D)t}{2} + \ldots \text{etc.}
\]

Where A, B, C, D, E and F represent the blood glucose increments, t represents the different time intervals between the blood samples, (Figure 1.4). (Wolever et al., 1991; Brouns et al., 2005).

\[
\text{GI} = \frac{\text{Incremental area under the curve for 50g available carbohydrate from test food}}{\text{Incremental area under the curve for 50g available carbohydrate from reference food}} \times 100
\]

Figure 1.5: Equation for the calculation of GI value for test foods. (Source Wolever et al., 1991).

1.6.2 The classification of the GI of foods

The GI value is a ranking system that indicates how quickly foods raise blood glucose levels; high GI foods are rapidly digested and absorbed by the small intestine and show a high glycaemic response, with their index between 70 and 100, when using a glucose
drink as a reference (index of 100) (Brouns et al., 2005). Conversely, low GI foods are digested and absorbed more slowly and show a low glycaemic response, with their index being less than 55 when compared to glucose as a reference. The index of medium GI foods is between 55 and 69 (Brand-Miller et al., 2003; Brouns et al., 2005; Kirpitch and Maryniuk, 2011). Overall this system is very useful for classifying different foods as it allows the releasable energy content of a diet to be calculated, and foods that release energy slowly can be selected to help maintain a feeling of fullness.

1.6.3 Factors influencing GI value of food

There are a number of factors that can affect the GI value obtained from a food, such as the combination of macronutrients used, the proportion of amylose and amylopectin, how the food is processed, and the starch type, the fibre in the food, the organic acids present, and the presence of simple sugars.

1.6.3.1 The combination of macronutrients

The presence of fat and protein in a carbohydrate meal can result in a different and variable glucose response, compared to carbohydrate alone, depending on the proportion of each nutrient in the meal (Pi-Sunyer, 2002). Ercan et al. (1994) investigated the effect of adding 50 g of fat, as butter, to a carbohydrate meal (which contained 50 g of available carbohydrate), on the glucose response, and found that the glucose response was significantly lower for the combination compared to the carbohydrate dose alone. Similarly, adding protein to a carbohydrate meal reduces the glycaemic response, possibly due to the protein having an effect on delaying gastric emptying and enhancing insulin secretion, which stimulates the peptide gut hormone release (Pi-Sunyer, 2002). Overall these results show that the carbohydrate content of a meal cannot be considered in isolation, the other components need to be considered in conjunction as they can affect its uptake.

1.6.3.2 Proportion of amylose to amylopectin

The nature of the starch in the carbohydrate meal is also important to consider in the calculation of the GI of foods. For most carbohydrate containing foods, the total starch can be divided up into 15-20 % amylose and 80-85 % amylopectin (Garrett and Grisham, 2012) (see section 1.5.2.2 and 1.5.2.3). The amount of amylose and amylopectin in the starch fraction is responsible for differences in rate of digestion,
which can be related to the difference in glycaemic response observed (Murray et al., 1998). This can be due to the differences in structure allowing glucose units of amylose to participate more in hydrogen bonding and the presence of less branched chains of glucose than amylopectin, making them less accessible to enzymatic digestion (Thorne et al., 1983; Murray et al., 1998). Furthermore, amylose has a lower molecular weight than amylopectin and a smaller surface area for enzymatic attack for amylose (O’Dea et al., 1980; Murray et al., 1998). Therefore, foods which are higher in amylose produce a lower GI (Pi-Sunyer, 2002); for example, Doongara rice, which has 28 % amylose, gave a lower GI (GI = 63), (P < 0.01) than normal rice (GI = 88), which has 20 % amylose (Miller et al., 1992). This shows that altering diets to contain higher quantities of amylose may help to maintain a sustained glucose release, reducing large spikes in blood glucose that can be detrimental.

1.6.3.3 Processing, preparation and cooking methods

The processing, preparation and cooking of foods can change the GI; grinding, rolling, and pressing can disrupt the granules of starch, which would allow more of the amylose and amylopectin macromolecules to be available for hydrolysis, and thus increase the GI of the food (Pi-Sunyer, 2002). Moreover, the cooking of starchy foods can produce a greater GI compared to the raw/uncooked foods, because cooking can increase the break-up of the starch granules, making them more readily available for hydrolysis. Studies have shown that starch granules can swell and burst when cooked, which also increases the availability of the starch to amylase (Thorne et al., 1983). These changes need to be taken into account when calculating GI and planning a diet to help weight loss or a controlled glucose release.

1.6.3.4 The physical form of the starch foods

Different physical forms of a single food, and particle size, can change the glycaemic response; for example, the GI of cooked ground rice was higher than cooked whole rice, when fed to both healthy people and diabetic patients. This is due to the fact that ground rice has a higher surface area than whole rice, and as such the rate of starch hydrolysis is increased (Thorne et al., 1983; Pi-Sunyer, 2002). A similar comparison can be drawn for fruit; for example, the GI for an orange is 42 ± 3, while that for orange juice is 52 ± 3. (Foster-Powell et al., 2002). This difference is due to the processing of the orange releasing the juice and the starches from the fruit.
1.6.3.5 Fibre type

Fibre includes dietary fibre and functional fibre; dietary fibre consists of non-digestible carbohydrate and lignin that are naturally occurring in foods, such as non-starch polysaccharides (NSP) which include cellulose, pectin and gum (Slavin, 2005). Functional fibre contains isolated non-digestible carbohydrates (Slavin, 2013). Dietary fibre can be further divided into soluble (viscous) and insoluble (non-viscous) fibre such as oat bran and guar gum (Slavin, 2005). Resistant starch (RS) is a constituent of fibre and occurs naturally in plant cell walls, it is resistant to enzymatic digestion, passing through the small intestine and reaching the large bowel (Bravo et al., 1998, Bagchi et al., 2000). RS can be separated into four classes: RS1 is a starch that is inaccessible to amylase digestion, RS2 is a starch that is accessible to the amylase by gelatinization, RS3 is an isolated fibre form that is created during cooking and cooling of starch rich foods, and RS4 is chemically modified starch not found in nature (Englyst et al., 1992; Cummings et al., 1996).

Soluble fibre reduces the glycaemic response by decreasing the absorption of macronutrients in the small intestine, as well as decreasing the availability of the starch for digestion (Wolever and Miller, 1995; Foster-Powell et al., 2002; Slavin, 2005). This occurs because the soluble fibre (especially non-starch polysaccharides) increases the viscosity of the food, which delays gastric emptying and intestinal absorption (Hallfrisch and Behall, 2000, Sasaki and Kohyama, 2012).

1.6.3.6 Organic acids

An organic acid is an organic compound with acidic properties, the most common are the carboxylic acids, such as citric acid found in fruit (Guevarra and Panlasigui, 2000). A high organic acid content in foods can lower the GI, for example, mango has a low GI because it contains malic, citric and tartaric acids, which could delay gastric emptying (Guevarra and Panlasigui, 2000). Moreover, increasing the amount of acidity of a meal has also been shown to reduce the GI of a meal (Pi-Sunyer, 2002). A study by Liljeberg and Björck (1998) showed that the presence of acetic acid (18 mmol per test meal) as vinegar reduced the postprandial blood glucose (GI = 64) and insulin indices (II = 65) significantly for a starchy meal, such as white wheat bread. This effect could be due to a delay in the gastric emptying rate (Liljeberg and Björck, 1998). This
suggests that adding these acidic fruits or acids such as vinegar, can make diets more effective by reducing the GI.

1.6.3.7 Anti-nutrients

Anti-nutrients are components that are present in some grains and legumes and can affect fasting glucose levels. These components include lectins, tannins and phytic acid, which is also known as myo-inositol hexaphosphate (Yoon et al., 1983; Greiner and Konietzny, 2006). Studies have shown that phytic acid added as a supplement in the diet can significantly reduce fasting glucose levels and haemoglobin A1c in diabetic KK mice (Lee et al., 2006). In addition, adding phytic acid to navy bean flour has been shown to decrease starch digestion \textit{in vitro} and GI \textit{in vivo} (Thompson et al., 1987). The mechanism responsible for this relationship is possibly that phytic acid may inhibit gastric emptying or inhibit starch digestion by altering the pH of the intestine (Yoon et al., 1983; Thompson et al., 1987). Another source of anti-nutrients are the polyphenols, such as tannins, which are widely present in foods such as legumes (Thompson et al., 1984). Polyphenols in legumes may have been shown to have the ability to reduce the glycaemic response to a carbohydrate food in healthy and diabetic people (Thompson et al., 1984). This effect may be due to the polyphenolic compounds inhibiting some enzymes, such as amylase, leading to reduced starch digestibility (Section 1.6) (Thompson et al., 1984; Siddhuraju et al., 2005; Williamson, 2013).

1.6.3.8 The presence of sugars

The common simple sugars present in foods are lactose, glucose and sucrose; they have different GIs, the GI of fructose is 19, glucose is 99, lactose is 46 and sucrose is 68, which shows how varied their response is (Foster-Powell et al., 2002). This shows that the GI value of foods is dependent on the type of sugar present, as fructose in a drink or meal produces a significantly lower glucose and insulin response compared with glucose and sucrose in a drink or meal (Wolever and Miller, 1995). For example, cake and ice-cream sweetened with fructose produces a lower glycaemic response than cake and ice-cream sweetened with sucrose (Wolever and Miller, 1995). This suggests that diets could be modified to include more fructose as this would help to reduce the GI.
1.6.4 GI and disease

Recently, many studies have suggested that low-GI foods may be beneficial for the prevention or treatment of a number of chronic diseases, including diabetes, CVD and cancer (Jenkins et al., 2002; Brand-Miller et al., 2003).

Low GI foods have a smaller 2 hour area under the glucose curve than white bread or glucose, which are used as controls, and this can lead to a reduced glycaemic and insulinaemic response; whereas high GI foods have a higher area under the glucose curve than the controls and lead to an increased insulin response (Bornet et al., 1987). Hodge et al. (2004) report that dietary high GI is positively associated with diabetes in a study of 31,641 type 2 diabetes participants completed at follow up. They suggest that a low GI diet may reduce the risk of type 2 diabetes. Another study examined the association between GI and dietary fibre in 91,249 women, who completed a frequency dietary intake questionnaire, and were followed for 8 years for the development of incident type 2 diabetes. This study found that high GI was significantly associated with an increased risk of diabetes (Schulze et al., 2004). McKeown et al. (2004) examined the association of insulin resistance, the prevalence of metabolic syndrome with dietary glycaemic index in 2,834 subjects. The outcome of their study showed that dietary high GI was positively associated with insulin resistance and the prevalence of metabolic syndrome. Several studies have shown that a high GI diet stimulates a greater level of insulin secretion than a low GI diet, which leads to hyperinsulinaemia, IR and increased beta cell demand (Jenkins et al., 1987, Miller, 1994, Ludwig, 2002). In addition, a high GI diet elevates blood glucose and free fatty acid levels that may impair beta cell function (Jenkins et al., 1987, Miller, 1994, Ludwig, 2002). However, there is evidence to suggest that low GI diets improve insulin sensitivity, and reduce the peak rise of blood glucose (Jenkins et al., 1987, Miller, 1994, Ludwig, 2002). Furthermore, they reduce blood glucose fluctuations during the day and this is one of the main benefits of consuming low GI foods, in particular for diabetic and pre-diabetic people who need to control their glucose levels (Jenkins et al., 1992, Ceriello, 1998). Studies have shown that a low GI diet can improve metabolic control in diabetes, and lower total plasma cholesterol and LDL cholesterol in type 2 diabetics, compared with a high GI diet, however, a low GI diet has no significant impact on HDL and triglyceride levels which is considered beneficial (Opperman et al., 2004, Kelly et al., 2004). On the other hand, a high GI diet has been shown to be strongly associated with an increased risk of
coronary heart disease (Liu et al., 2000). It is clear that there are several health benefits in using GI as an indicator for daily diets, as it as can help to reduce the risk of disease.

1.7 Polyphenols

Polyphenols are a group of phytochemicals widespread in plants; they are present in foods such as fruits, vegetables, herbs, and cereals and they are essential to the physiology of plant function, structure and growth (Haslam, 1998). There are more than 8000 phenolic structures currently known, which can be simple molecules such as phenols, or more complex and variable in their composition, such as tannins (Harborne, 1993; Bravo, 1998; Urquiaga and Leighton, 2000). Simple phenols (Figure 1.6 A), polyphenols (Figure 1.6 B) and tannins that are present naturally in food have been used for many years to give foods and beverages specific colours, odours and flavours (Shahidi and Naczk, 1995). Polyphenol structures consist of several hydroxyl groups on two or more six-carbon aromatic rings (D’Archivio et al., 2007; Stevenson and Hurst, 2007; Lindsay and Clifford, 2000). Therefore, molecules with only one ring, such as phenolic acids and phenolic alcohols, are strictly not polyphenols, however they share many properties and characteristics of polyphenols and so are usually grouped together when considering the properties of the components of plants, foods and beverages (Stevenson and Hurst, 2007).

![Figure 1.6: A) Basic structure of phenol B) Basic structure of polyphenols](image)

1.7.1 Classification of polyphenols

Polyphenols can be divided into different classes depending on their structure and function (Clifford, 2004; Weichselbaum and Buttriss, 2010). The main groups of polyphenols are flavonoids, phenolic acids and tannins (Figure 1.7); flavonoids can be further divided into several subclasses which are: flavones, flavanones, flavonols, flavanols (also called flavan-3-ols), anthocyanidins and isoflavones (D’Archivio et al., 2007; Opara and Chohan., 2014).
Tannins can also be further divided into two groups, which are the condensed (proanthocyanidins) and hydrolysable tannins. Moreover, any polyphenols that have higher molecular weight structures (molecular weights ≥ 500) are usually classified as tannins (Khandelwal et al., 2010).

Figure 1.7: The classification of polyphenols (source Opara and Chohan., 2014).

1.7.2 Proanthocyanidins

Proanthocyanidins (PAs) are oligomeric and polymeric flavan-3-ols, a type of tannin; they are present in fruits and vegetables, and the bark, leaves and seeds of many plants. They play an important role in plants as they provide protection against predation, and they also can give flavour to beverages such as teas (Dixon et al., 2005). They share some common structural features with phenols such as procyanidin. Little is known about their dietary intakes, although they are likely to contribute to a large part of the daily polyphenol consumption due to their abundance in commonly consumed plants (Santos-Buelga and Scalbert, 2000). There are two main types of
PAs in fruits, vegetables, and herbs, A-type procyanidin contains (+) – catechin and/or (-) epicatechin units that are doubly link through carbon C2→C7 and C4→C8 bonds (Figure 1.8 A), whereas B-type procyanidin contains a single link through C4→C8 bond (Figure 1.8 B) (Passos et al., 2007). A-type trimers and tetramers have molecular masses of 864 and 1152 Da, respectively, whereas, B-types have trimers and tetramers with molecular masses of 866 and 1154 Da, respectively (Lu et al., 2011).

The absorption, distribution, metabolism and excretion of proanthocyanidins is based on their chemical structures (Bravo, 1998). The absorption of proanthocyanidins is minor (Holt et al., 2002). Proanthocyanidins with a degree of polymerisation (DP) less than 3 are depolymerised into mixtures of epi-catechin or catechin monomers and dimers are absorbed by the small intestinal. While proanthocyanidins DP more than 10, move from the small intestine to the large intestine and are degraded by micro-flora (Kruger et al., 2014). Although proanthocyanidins show impaired absorption in the small intestine, they still have health benefits (Teixeira, 2002). Proanthocyanidins such as procyanidin B2 and procyanidin A are metabolized by gut flora to produce phenolic acids, which can be detected in urine, and other metabolites are also formed. These may be important in terms of health effects in the gut (Forester and Waterhouse, 2009). There are a variety of gut flora that could participate in the metabolism of polyphenols, in particular the flavonoids, these are Bacteroides spp. and Eubacterium spp. (Blaut and Clavel, 2007).

![Figure 1.8](image-url): A-type procyanidin (A) and B-type procyanidin structure (B), (Passos et al., 2007).
1.7.3 The health properties of polyphenols

Studies have suggested that polyphenols might be important for health as they have a higher antioxidant activity than vitamin E and also carotenoids in vitro (Rice-Evans et al., 1997). They have been shown to inactivate free radicals, such as lipid peroxides, and they can reduce the oxidative effects of metal ions in vitro (Rice-Evans et al., 1997). Furthermore, there is evidence that they are able to inhibit pro-oxidant enzymes and enhance antioxidant enzymes in vitro, and also protect other antioxidants, such as ascorbic acid, against oxidation in vitro (Aron and Kennedy, 2008; Heinonen, 2007; Stevenson and Hurst, 2007; Saura-Calixto et al., 2007; Lopez-Lazaro, 2009).

However, the impact of polyphenols on antioxidant capacity in vivo has been shown to be different to that in vitro; D’Archivio et al. (2007), Stevenson and Hurst (2007) and Ghosh and Scheepens (2009) have stated that polyphenols do not appear to be circulating in the blood at high enough concentrations to contribute significantly to the antioxidant capacity of the body. Studies have also shown that approximately 90-95% of the polyphenols ingested undergo molecular changes in their structure, and as such changes in their biological activities, compared to polyphenols investigated in vitro studies (Lotito and Frei, 2006; Stevenson and Hurst, 2007). Most polyphenols that are present in plants in the form of esters, glycosides or polymers are not absorbed, therefore these compounds, once ingested, travel to the large intestine where they are broken down by intestinal enzymes or microflora in the colon, which would modify the polyphenols allowing them to be absorbed (D’Archivio et al., 2007; Saura-Calixto et al., 2007; Aron & Kennedy, 2008). A study by Visioli et al. (2009) claimed that the polyphenol forms reaching the blood and tissues were different from those present in food which concurs with other research in this area. The rate of polyphenol absorption is determined by the structure of the polyphenols, not by their concentration, and in addition, some of the variation observed for polyphenol absorption and metabolism may be due to the differences in the composition of the gut microflora between patients (D’Archivio et al., 2007; Weichselbaum and Buttriss, 2010). Overall this suggests further research is required to determine which factors play an important role in their uptake.

There are many health benefits associated with the consumption of polyphenols, for example the consumption of plants which are high in polyphenol content, such as fruits
and vegetables, is associated with a reduced risk of CVD and an improvement in vascular endothelial function (Barona et al., 2012; Hodgson et al., 2006). Furthermore, studies have suggested that there can be a beneficial modification of the hormonal profile, modification of the lipid profile, a decrease in serum cholesterol and an increase in insulin sensitivity, after consumption of a polyphenol-rich diet, which may be due to more than just the polyphenols in the diet (Goldberg, 2003; Stanner, 2005; Kar et al., 2006; Stevenson and Hurst, 2007; Aron and Kennedy, 2008; Ghosh and Scheepens, 2009). Polyphenols are considered as non-nutrients; however, they have been shown to have antioxidant and anti-inflammatory effects in vitro (Bravo, 1998; Harborne, 1993).

Consuming fruits, vegetables and herbs, which are rich in polyphenols, might also contribute to reducing the risk of some forms of cancer, such as mouth, oesophagus, stomach and lung (WCRF/AICR, 2007). Herbal medicines such as cinnamon are considered as a good source of polyphenols (Anderson et al., 2004). These compounds are of interest as research has shown they are one group of the active compounds in cinnamon, and may be responsible for the medicinal properties observed.

1.7.4 Polyphenols in cinnamon

Studies on the composition of cinnamon using LC-MS have shown that about 26.8% of crude cinnamon extract is condensed tannins, 23.2% is proanthocyanidins, and 3.6% epi-catechins, which shows that cinnamon has a high polyphenol content (Shan et al., 2007). There are two major types of proanthocyanidins present in cinnamon which are A-type procyanidin and B-type procyanidin (section 1.6.1.1) (Jarvill-Taylor et al., 2001; Lu et al., 2011).

A study by Anderson et al. (2007) has indicated that A-type procyanidins perform an insulin like activity in vitro, which suggests they could be used to help patients with diabetes. Furthermore, a study by Lu et al. (2011) confirmed that A- and B-type procyanidins analysed by LC-MS methods exist in cinnamon, which might have hypoglycaemic activities and may improve insulin sensitivity. The effect of co-administration of C. cassia extracts at doses of 200 and 300 mg/kg body weight with a high-fat diet to male KM mice for 14 days has been studied. The results showed that
blood glucose concentrations were significantly decreased in animals fed cinnamon extract compared with the control group (Lu et al., 2011).

Another study has shown that cinnamon extracted with acetone has antioxidant activity as shown in the linoleate oxidation assay and the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay in vitro (Jayaprakasha et al., 2007). In a recent review of bioactive properties of herbs and spices, cinnamon was demonstrated to have a significant inhibitory action on the pro-inflammatory enzyme cyclo-oxygenase-2 (COX-2), which is probably related to the polyphenolic content of cinnamon, as these type of polyphenols have previously been shown to inhibit this enzyme (Opara and Chohan, 2014). Cinnamon also decreases lipopolysaccharide (LPS) TNF-α stimulation in mice serum, and it inhibits the generation of TNF-α and IL-6 in male BALB/c mice, which shows that cinnamon can cause immune modulation. This anti-inflammatory activity of cinnamon is related to the presence of polyphenols in cinnamon (Hong et al., 2012). Furthermore, catechin, epicatechin and procyanidin B2, which have been identified in cinnamon extract, have been shown to inhibit the formation of advanced glycation end-products (AGEs) in vitro (Peng et al., 2008). Accumulation of AGEs are seen in the pathogenic process, and are associated with many disease states such as diabetes, Alzheimer’s disease and CVD (Peng et al., 2008). Further evidence of the benefits of cinnamon have come from a study by Kannappan, et al. (2006) which found that an aqueous cinnamon extract (100 mg/ml) improved glucose metabolism and prevented hyperlipidaemia in fructose-fed male albino rats. A study by Cao et al. (2007) summarised the potential effect of cinnamon polyphenols (CP) in the insulin signalling pathway in type 2 diabetics (Figure 1.9); CP activated insulin receptors by increasing their tyrosine phosphorylation activity and by decreasing phosphatase activity that inactivates them, moreover, CP increased the concentration of the insulin receptor β, GLUT4 protein, glycogen synthase activity and glycogen accumulation (Cao et al., 2007). Overall this study showed that CP have many effects on the insulin signalling pathway, suggesting it may be helpful for people with diabetes.
Figure 1.9: A model of the pathways contributing to the beneficial effects of cinnamon polyphenols in insulin signalling (Cao et al., 2007). Cinnamon polyphenols (CP) enhance insulin receptors by increasing their tyrosine phosphorylation activity. CP increased the concentration of the insulin receptor β, GLUT4 protein, glycogen synthase activity and glycogen accumulation. CP reduced COX-2 and cytokines such as TNF-α.

1.7.5 Cinnamaldehyde in cinnamon

Cinnamaldehyde (cinnamic aldehyde) is one of the principal volatile oil components in cinnamon that gives the cinnamon its particular flavour (Rao and Gan, 2014) (Figure 1.10). The properties of this oil have been investigated in a study by Subash Babu et al. (2007) who reported that the cinnamaldehyde in cinnamon had hypoglycaemic and hypolipidaemic effects in male diabetic Wistar rats. The doses administered in the study were 5, 10 and 20 mg/kg body weight (BW) for 45 days. The highest dose of 20 mg/kg BW, significantly decreased glycosylated hemoglobin (HbA1c), serum total cholesterol and triglyceride levels. This study shows the potential effects that could be achieved in humans and the doses that would be useful in future studies.
There are many studies that show cinnamon is an effective herbal medicine with many health benefits in *vitro* and in animal studies, however, only a few randomised human studies have highlighted the effect of cinnamon on glycaemia, which limits the conclusions that can be drawn about the potential health benefits of cinnamon in diabetic and pre-diabetic subjects (Gruenwald et al., 2010). Also, the anti-inflammatory property of cinnamon has been confirmed *in vitro* (Lee et al., 2005; Opara and Chohan, 2014) but no human studies have been conducted, therefore it is worth examining the anti-inflammatory, anti-diabetic and lipid reducing properties in humans through studying the effect on biomarkers, inflammatory markers such as cytokines, adhesion molecule markers, glycaemic control, and lipid profiles.

### 1.8 Cytokines

Cytokines are very low molecular weight compounds, and they are involved in the regulation of the immune system and in the intercellular signalling response (Bown et al., 2001). Many different cell types produce cytokines and regulate the proliferation and differentiation, and other activities of cells (Scheller et al., 2011). Cytokines can be divided into those that are primarily pro-inflammatory and those that are anti-inflammatory; pro-inflammatory cytokines promote the production of further cytokines and cause inflammation; these include interleukin 1 (IL-1), interleukin 2 (IL-2), interleukin 6 (IL-6) and interleukin 8 (IL-8), tumour necrosis factor alpha (TNF-α) and Interferon gamma (IFN-γ) (Souza et al., 2008). Anti-inflammatory cytokines are able to inhibit macrophage function and can act indirectly on T cells, for example IL-4, IL-10, and IL-13, and they lead to a reduction in pro-inflammatory cytokine production (Souza et al., 2008; Bown et al., 2001). All cytokines have multiple biological activities in multiple cell types, they can show stimulatory or inhibitory activities of other cytokines and hormones (Matsumoto and Kanmatsuse, 2000). Cytokines play important roles in
repairing damaged tissue, controlling cell replication and apoptosis, and also in the modulation of immune reactions (Foster, 2001). In acute and chronic inflammatory diseases in humans, such as arthritis, a large concentration of cytokines are released into plasma (Arvidson et al., 1994; Vindenes et al., 1998). Furthermore, monitoring the change in cytokine levels can be used as an indirect measurement of the progression of the disease in response to therapy, such as antibiotics, for example CRP levels can be monitored over time (Zissel et al., 1999; Simpson et al., 2000). Research by Dinarello (2000) has suggested that the susceptibility to disease is genetically determined by the balance or expression of either pro-inflammatory or anti-inflammatory cytokines. Pro-inflammatory cytokines promote inflammation and infection such as IFN-γ that augments TNF-α activity and induces nitric oxide (NO), whereas anti-inflammatory cytokines suppress the intensity of the cascade of pro-inflammatory cytokines or block this process (Dinarello, 2000). The balance between pro-inflammatory and anti-inflammatory cytokines can determine the outcome of disease, whether in the short term or long term (Dinarello, 2000).

A study by Hatanaka et al. (2006) found that there was an excessive release of pro-inflammatory cytokines (namely IL-8, IL-1β and TNF-α) produced by neutrophils and monocytes associated with type 2 diabetic patients, which suggests that there is an immune role in the progression of diabetes that could be treated to reduce symptoms. Obesity is also associated with a state of chronic inflammation, the detection of an elevated release of pro-inflammatory cytokines from obese adipose tissue was the first evidence of a direct connection between obesity and systemic inflammation (Berg and Scherer, 2005). This study showed that CRP concentration, which is considered as a systemic inflammation marker, was increased in obese patients (Berg and Scherer, 2005). Further evidence of an immune role comes from the fact that adipose tissue of obese patients increases the expression of pro-inflammatory proteins such as ICAM and MCP-1 (Terra et al., 2009).

1.8.1 The cytokine families

There are six families of cytokines:

1- The IL-1 family are soluble cytokines that are secreted very early in the immune response by dendritic and endothelial cells, as well as monocytes and macrophages,
and include IL-1α and IL-1β. They are considered to be pro-inflammatory (Hamblin, 1993; Thomson and Lotze, 2003).

2- The hematopoietin Class I cytokine family are soluble cytokines that communicate between immune cells and are secreted by T cells, B cells, mast cells, endothelial cells, monocytes, and macrophages. Their name is derived from the function of some of these cytokines, which are involved in the formation of blood cells. IL-2, IL-4, IL-6 and IL-12 are examples of this family (Hamblin, 1993; Thomson and Lotze, 2003).

3- The TNF family are soluble cytokines that regulate the development, function and homeostasis of skeletal cells, for example TNF-α and TNF-β. They are secreted from macrophages, T cells, B cells and natural killer cells (Hamblin, 1993; Thomson and Lotze, 2003).

4- The IL-17 family, for example IL-17A and IL-17B, have been recently discovered and are considered to be pro-inflammatory. They play important roles in protective mechanisms against fungal and bacterial infections, and development of autoimmunity and inflammation. They are regulated by the innate and adaptive immune system (Hamblin, 1993; Thomson and Lotze, 2003; Iwakura et al., 2011).

5- The IFN Class II cytokine family are regulated and modulated by the immune response, they are released from leucocytes, fibroblasts, natural killer cells, T cells and endothelial cells. This family includes IFN-α, IFN-β, IFN-γ, and IL-10. The interferon family play an important role in controlling virus infection (Hamblin, 1993; Thomson and Lotze, 2003; Randal and Goodbourn, 2008).

6- Chemokines are a type of cytokine which produce a chemical that attracts and directs other immune cells to migrate to the site of inflammation, or tissue damage, and these regulate the immune cell function. They are released from monocytes, endothelial cells, epithelial cells, and fibroblasts. This family includes IL-8 and monocyte chemoattractant protein-1 (MCP-1) (Hamblin, 1993; Thomson and Lotze, 2003).

The cytokines are potent mediators of inflammatory processes and the maintenance of a homeostatic balance; they can work together in an inflammatory cascade (Figure 1.11) and could potentially be used as predictors of the prevalence or incidence of some diseases, such as CVD (Pearson et al., 2003).
Figure 1.11: The relationship of inflammatory markers and some diseases, such as CVD (adapted from Pearson et al., 2003).

1.9 Adhesion molecules

There is evidence to suggest that within diseases cell adhesion molecules (CAMs) can play a vital role in inflammation. CAMs are a group of glycoproteins; they are expressed by several cell types including leukocytes and endothelial cells (Lawson and Wolf, 2009). Endothelial cells are the inter cell line of the circulation system, which are specialise to different cell types, such as arterial and venous systems (Marcelo et al., 2013). CAMs are constitutively present on endothelial cells and they include three families which are the selectins, integrins, and the immunoglobulin (Ig) superfamily (Furie, 2014). The expression of CAMs are increased by pro-inflammatory cytokines, and CVD (Lawson and Wolf, 2009). Adhesion molecules have also been shown to be involved in the recruitment and migration of leukocytes and neutrophils to sites of inflammation (Lawson and Wolf, 2009; Furie, 2014). The migration of neutrophils involves multiple steps and in each step a different family of adhesion molecules takes
part; for example, the rolling phase is mediated by the selectin family, and activation of neutrophil migration to the endothelium is regulated by integrins and the Ig superfamily (Etzioni, 1996).

1.9.1 Immunoglobulin (Ig) superfamily

Intercellular adhesion molecule-1 (ICAM-1) is a member of the Ig family and is expressed by several cell types including leukocytes and endothelial cells. Studies have shown that its expression is increased in CVD, autoimmune disorders, cancer, and other diseases (Lawson and Wolf, 2009; Furie, 2014). These results show this is an important marker to measure when determining disease progression, and also for the effectiveness of any treatments used.

Vascular cell adhesion molecule-1 (VCAM-1) is a member of the Ig family and is expressed by endothelial cells. There is evidence to suggest that it is induced during inflammation (Furie, 2014). This suggests it would be a good marker to measure to determine the level of inflammation in diseases and how this changes during treatment.

Studies have shown that the adipose tissue of obese people can increased the expression of pro-inflammatory proteins, such as ICAM and VCAM (Terra et al., 2009). It has been suggested that the elevated concentrations of adhesion molecules play a role in the formation and progression of atherosclerosis (Turan et al., 2014). Therefore, losing weight may prevent the progression of atherosclerosis in the overweight and obese population as they will have less adipose tissue producing the detrimental factors (Poirier, 2002).

1.9.2 Selectins

Selectins are transmembrane molecules that bind with carbohydrate ligands. There are three members of this family: E-selectin, P-selectin and L-selectin; E-selectin is expressed by endothelial, whereas P-selectin is produced by platelets, and L-selectin is formed on leukocytes and lymphocytes. They mediate the cell interaction of recruitment of leukocytes into endothelium (Furie, 2014). These molecules can play an important role in the progression of disease as a reduction or an increase in the number of leukocytes can cause different outcome.
1.10 C-reactive protein (CRP)

One of the key systemic inflammation markers is CRP, which is synthesised by the liver and regulated by IL-6 (Otsuka et al., 2014). An increased CRP level is associated with many diseases such as CVD, obesity and IR (Yudkin, et al., 1999). In healthy and lean people the concentration of CRP increases 2000-fold during the first 24–48 h after tissue injury and inflammation (Gaysina et al., 2011). The approximate levels of CRP in a normal adult population is (< 1 mg/l), average low risk (1.0-3.0 mg/l) and high risk at (> 3.0 mg/l) (Ridker, 2003). Higher CRP levels, which are above 3 mg/l, have been shown to be associated with metabolic syndrome, cardiovascular disease, diabetes and obesity (Kritchevsky et al., 2005; Calabrò et al., 2010; Gaysina et al., 2011; Otsuka et al., 2014). The higher levels of CRP show that in these diseases there is an increase in inflammation which can cause some of the symptoms associated with this disease states.

1.11 Inflammatory markers

Inflammatory marker levels play a role in predicting the incidence of some diseases such as cardiovascular disease. Inflammatory markers are used to detect acute inflammation, which might reveal a specific disease, and to give a marker of treatment response (Watson, 2012). In 2002 a workshop was organised by the American Heart Association and the Centers for Disease Control, title “CDC/AHA Workshop on Inflammatory Markers and Cardiovascular Disease: Applications to Clinical and Public Health Practice”, to make recommendations on how inflammatory markers should be used with other assessments of cardiovascular risk (Pearson et al., 2003). This workshop highlighted that determination of CRP could add value in predicting coronary events, and recommended in general population screening that CRP might be useful in predicting risk of coronary heart disease. Kritchevsky et al., (2005) stated that other inflammatory markers such as IL-6 and TNF-α may be useful markers for indicating elevated risk of cardiovascular disease. In addition, the concentration of inflammatory markers may indicate both the acute inflammatory state and long-term risk of many diseases, however, more work is needed to understand the ability of markers to predict over a longer periods of time in older populations (Kritchevsky et al., 2005).

The normal serum levels of soluble cytokines, adhesion molecules and CRP in the healthy population have been studied by several groups including the Randox
laboratory (Table 1.4, Table 1.5, and Table 1.6), and show how much variation there is between different age groups and using different detection methods (Kim et al., 2011; Kleiner et al., 2013). These values provide a starting point for evaluating how different diseases affect the expression of these markers, and also how effective the treatments for the diseases are.

**Table 1.4** The serum levels of soluble cytokines in healthy subjects as measured by the Randox laboratory, Kim et al. (2011) and Kleiner et al. (2013) when investigating.

<table>
<thead>
<tr>
<th>References</th>
<th>Randox laboratory</th>
<th>Kim et al., 2011</th>
<th>Kleiner et al., 2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>Age 20-45</td>
<td>45 &gt; Age ≥ 65</td>
<td>Age ≥18</td>
</tr>
<tr>
<td>Cytokines</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-2 (pg/ml)</td>
<td>0.00 - 2.42</td>
<td>5.58±4.17(3.06-32.1)</td>
<td>14 (9.4–15.9)</td>
</tr>
<tr>
<td>IL-4 (pg/ml)</td>
<td>0.88 - 2.63</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>0.00 - 2.13</td>
<td>2.57±5.22 (0.16-31.5)</td>
<td>ND</td>
</tr>
<tr>
<td>IL-8 (pg/ml)</td>
<td>0.95 - 14.11</td>
<td>27.6±43.9(4.76-217.0)</td>
<td>29.3 (24.4–35.9)</td>
</tr>
<tr>
<td>IL-10 (pg/ml)</td>
<td>0.04 - 1.05</td>
<td>1.58±6.17(0.01-41.7)</td>
<td>12.6 (8.5–16.7)</td>
</tr>
<tr>
<td>VEGF (pg/ml)</td>
<td>0.00 - 211.65</td>
<td>100.5±75.4(6.9-329.3)</td>
<td>61.6 (32–118.9)</td>
</tr>
<tr>
<td>IFN γ (pg/ml)</td>
<td>0.00 - 1.24</td>
<td>10.3±18.4(1.09-117.7)</td>
<td>ND</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>2.28 - 7.81</td>
<td>4.94±4.79(0.86-20.8)</td>
<td>ND</td>
</tr>
<tr>
<td>IL-1β (pg/ml)</td>
<td>0.00 - 2.34</td>
<td>2.52±7.41(0.17-39.0)</td>
<td>ND</td>
</tr>
<tr>
<td>IL-1α (pg/ml)</td>
<td>0.00 - 0.27</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>MCP-1 (pg/ml)</td>
<td>44.62 - 395.52</td>
<td>168.0±73.0(39.3-355.9)</td>
<td>41.5 (20.1–78.9)</td>
</tr>
<tr>
<td>EGF (pg/ml)</td>
<td>54.06 - 196.88</td>
<td>61.0±65.1(3.20-251.6)</td>
<td>ND</td>
</tr>
</tbody>
</table>

*The levels of the cytokines, chemokines, and growth factors were analysed using The Evidence Investigator™ Cytokine & Growth Factors High-Sensitivity immunoassays based on biochip.*

b*The levels of the cytokines, chemokines, and growth factors were analysed using multiplexed bead-based immunoassays. Data present as mean range or mean± standard deviation. ND=not determined*
Table 1.5 The serum levels of CRP in healthy subjects as measured by the Randox laboratory and Ponthieux et al. (2004).

<table>
<thead>
<tr>
<th>References</th>
<th>Randox laboratory a</th>
<th>Ponthieux et al., 2004 b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>Age 20-45</td>
<td>Age 20-45</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>0-1</td>
<td>1.64 ± 2.32</td>
</tr>
</tbody>
</table>

a The levels of CRP was determined by Immunoturbidimetric assay ILAB 600.

b Immunonephelometry on a Behring Nephelometer Analyser. Data present as mean range or mean± standard deviation

Table 1.6 The serum levels of soluble adhesion molecules in healthy participants according to, Nash et al. (1996), Zonneveld et al. (2014), and Randox laboratory.

<table>
<thead>
<tr>
<th>References</th>
<th>Randox laboratory a</th>
<th>Nash et al., 1996 b</th>
<th>Zonneveld et al., 2014</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>Age 20-45</td>
<td>Age 20-45</td>
<td>Age 20-71</td>
</tr>
<tr>
<td>Adhesion molecules</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VCAM-1 (ng/ml)</td>
<td>260.07 - 632.54</td>
<td>450-800</td>
<td>569±98</td>
</tr>
<tr>
<td>ICAM-1 (ng/ml)</td>
<td>147.14 - 299.55</td>
<td>150-300</td>
<td>208± 20.5</td>
</tr>
<tr>
<td>E-selectin (ng/ml)</td>
<td>4.92 - 27.68</td>
<td>20- 60</td>
<td>42± 19.4</td>
</tr>
<tr>
<td>P-selectin (ng/ml)</td>
<td>92.64 - 261.43</td>
<td>ND</td>
<td>116.9 ± 33.4</td>
</tr>
<tr>
<td>L-selectin (ng/ml)</td>
<td>876.78 - 1811.87</td>
<td>ND</td>
<td>1600± 800</td>
</tr>
</tbody>
</table>

a The levels of adhesion molecules were analysed using Evidence Investigator™ Biochip Array Technology that based on immunoassays

b The levels of adhesion molecules were analysed using sandwich ELISA system

Data present as mean range or mean± standard deviation. ND=not determined

1.12 Aims of the thesis

This PhD research aims to investigate the hypothesis that consuming cinnamon supplements may improve glycaemia, lipid profiles, weight, blood pressure, insulin resistance and inflammatory markers in an overweight population. To test this hypothesis and to achieve this overall aim, a series of in vitro and human studies were conducted:

Objective 1: In vitro studies

The in vitro work consisted of two studies, which aimed to:
1. To optimise the extraction of polyphenols from cinnamon.
2. To determine the total phenol and tannin content of a variety of cinnamon supplements.
3. To determine the phenolic composition of *C. cassia* and *C. zeylanicum* using LC-MS.
4. To test the antioxidant properties of *C. cassia* and *C. zeylanicum* supplements and the free-radical scavenging capacity of cinnamon types.
5. To test the effect of *C. cassia* and *C. zeylanicum* supplements on RAG and SAG values.

**Objective 2: In vivo human studies**

The human studies involved the assessment of the acute effects of *C. cassia* supplementation in a healthy population and a longer term (8 week) evaluation of consuming *C. cassia* supplementation in overweight healthy women 45-70 years with the following aims:

**Acute effects**

1- To examine the effect of *C. cassia* supplementation (1 g) on glycaemic response.

**Long term effects**

1- To examine the effect of 4 and 8-week *C. cassia* supplementation (5 g) on weight, BMI, waist circumstance, hip circumference and heart rate.
2- To examine the effect of *C. cassia* supplementation (5 g) on fasting insulin and glucose levels.
3- To examine the effect of *C. cassia* supplementation (5 g) on oral glucose and insulin tolerance tests.
4- To examine the effect of *C. cassia* supplementation (5 g) on insulin resistance and HOMA.
5- To examine the effect of *C. cassia* supplementation (5 g) on CRP levels.
6- To examine the effect of *C. cassia* supplementation (5 g) on the levels of specific cytokines: IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, EGF, IFN-γ, MCP-1, TNF-α, and VEGF.
7- To examine the effect of cinnamon cassia supplementation (5 g) on the levels of adhesion molecules: VCAM, ICAM, E-selectin, P-selectin, and L-selectin.

8- To examine the correlation between the inflammatory markers and bone density in overweight healthy women aged 45-70 years.
CHAPTER TWO
Chapter 2

Materials and methods

The following chapter will describe the materials and methods used to carry out the experimental work in this thesis. Individual protocols for each experiment or study will be described in detail at the beginning of each chapter.

2.1 Materials

2.1.1 Equipment

1. Abbott Laboratories Ltd., Witney, Oxfordshire, UK:
Freestyle Optium.

2. Alpha Laboratories Ltd., Eastleigh, UK:
Apex Plus Flat Screw Cap Rad; Apex Plus Flat Screw Cap Blue; Micro-centrifuge tube, Skirted (1.5 ml).

3. Becton Dickinson (BD) and company, Oxford, UK:
Vacutainer blood collection tubes: sodium fluoride tubes (2 ml); EDTA tubes (4 ml); serum tubes (6 ml); Lithium Heparin tubes (4 ml); BD Vacutainer single use holder; BD Vacutainer safety-lok blood collection set 21G 18 cm (green).

4. CapsulCN International Co. Ltd., USA. Halal Capsules size 000 dark green (bovine gelatin), CapsulCN International Co. Ltd., USA.


6. Fisher Scientific Ltd., Loughborough, Leicestershire, UK:
Pipette; pipette tips; plastic tubes; Falcon™ tubes (50 ml), conical flask (250 ml); Pyrex™ beakers (10-2000 ml); 1.5 ml plastic cuvettes; filtering flask; filter disk.

7. Fisons Ltd., Leicestershire, UK:
Vortex-mixer (WhirliMixer).
8. Grant Instruments Ltd., Cambridge, UK:
Water bath with temperature range 35-90 °C (Grant Y22); shaking water-bath, with maximum shaking capacity of not less than 160 strokes per minute and a stroke length of 35 cm, temperature range 35-70 °C (Grant SS40-D).

9. Idass, Kettering, Northamptonshire, Ltd., UK:
Tape to measure the waist and hip circumferences of the participants.

10. Instrumentation Laboratory, Birchwood Science Park, Warrington, UK:
ILab 650 Clinical Chemistry System.

11. KNF Neuberger Inc., Trenton, USA:
Vacuum pump, LABOPRT.

12. Kontron Ltd., UK:
Uvikon 860 Spectrophotometer.

13. Mettler Toledo Ltd., Leicestershire, UK:
Digital scale, sensitivity ± 0.1 mg (AL 204); pH meter (SevenEasy).

14. OMRON Healthcare Europe BV, Hoofddorp, Netherlands:
Automatic blood pressure device (Omron MX3 Plus).

15. Owen Mumford Ltd., Oxford, UK:
Unistik 3 extra single use lancets.

16. Randox Laboratories Ltd., Crumlin, UK:
Evidence Investigator.

17. Sarstedt Ltd., Leicester, UK:
300 µl fluoride oxalate microvette tubes, 300 µl plasma plain microvette tubes.

18. Seca Ltd., Birmingham, UK:
A standard stadiometer to measure the participant’s height in centimetres.

19. Sigma Chemical Company Ltd., Poole, UK:
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Centrifuge 6K10.

20. Stratec Medizintechnik GmbH, Pforzheim, Germany:
Peripheral Quantitative Computed Tomography (pQCT) scanner.

21. Stuart Scientific Ltd., Staffordshire, UK:
Magnetic stirrer (SMS).

22. Tanita TBF-300, Tanita UK Ltd., Middx, UK:
Tanita scales to measure the weight and BMI of the participants.

23. Wallac Wizard, Wallac International, Finland:
Wallac 1470 wizard automatic gamma counter; Wallac 1410 liquid scintillation counter.

24. Whatman International, Maidstone, Kent:
Whatman No.1 filter paper.

25. YSI Life Sciences, Hampshire, UK:
YSI 2300 STAT Plus glucose analyser; YSI analyser printer paper.

2.1.2 Cinnamon supplements

This study focused only on the cinnamon supplements that were available to buy in health shops and were consumed as capsules.


- Cinnamon bark (Health Aid® Cinnamomum zeylanicum) 850 mg, Health Aid Limited, Middlesex, England, UK. (C. zeylanicum. E). Ingredients for each capsule: cinnamon powder (Cinnamomum zeylanicum), vegetable hydroxypropyl methylcellulose (HPMC), di-calcium phosphate, cinnamon extract, silicon dioxide, vegetable magnesium stearate. (C. zeylanicum. H).

- Cinnamon bark (Puritan's Pride's® Cinnamomum cassia) 500 mg, Holbrook, USA. Ingredients for each capsule: pure Cinnamomum cassia powdered bark, vegetable hydroxypropyl methylcellulose (HPMC). (C. cassia).
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- Cinnamon bark (Solgar® *Cinnamomum cassia*) 500 mg, Solgar Vitamin and Herb, USA. (*C. cassia*. E). Ingredients for each capsule: cinnamon powdered bark, cinnamon bark extract, silicon dioxide, vegetable magnesium stearate, vegetable hydroxypropyl methyl cellulose (HPMC), cellulose gum. (*C. cassia*. S).

### 2.1.3 Chemical and reagents

All chemicals were of analytical grade unless otherwise stated.

1. Englyst Carbohydrate Services Ltd., Southampton, UK:
   Amyloglucosidase (from *Aspergillus niger*, 10000 U/ml in glycerol; 1 unit releases 1 µmol glucose pH4.5, 55 °C), Englyst Cornflakes reference.

2. Fisher Scientific Ltd., Loughborough, Leicestershire, UK:
   Calcium chloride dihydrate (CaCl$_2$.2H$_2$O); ethanol; methanol; potassium chloride (KCl); potassium hydroxide (KOH); invertase (general purpose grade, from yeast); sodium chloride (NaCl); sodium hydroxide (NaOH); sodium acetate (trihydrate); trichloroacetic acid.

3. Merck Millipore GmbH, Darmstadt, Germany:
   Immuno-chemiluminometric insulin assay (Millipore Insulin kit, HI-14K).

4. Randox Laboratories Ltd., Crumlin, UK:
   Glucose GOD/PAP (glucose oxidase/phenol and 4 amino-antipyrine) test kit; non-esterified fatty acid (NEFA) kit; triacylglycerides (TAG) kit; chemistry control level 1 and 2; cytokine high sensitivity array, Biochips (cytokine); adhesion molecule array, Biochips (adhesion molecule); high sensitivity C reactive protein (hs-CRP); total cholesterol, high-density lipoproteins (HDL). Reagent signal EV805 for cytokine and adhesion molecules, Luminol EV805 and peroxide were mixed (1:1 v/v); cytokine high sensitivity diluent assay (20 mM, pH 7.2); cytokine high sensitivity conjugate (20 mM, pH 7.5); cytokine high sensitivity calibration: 2 ml of deionised water was added to each of the nine calibration bottles and rolled on a machine for 30 minutes; adhesion molecule calibration: 1 ml of deionised water was added to each of the nine calibration vials and rolled on a machine for 30 minutes; adhesion molecule diluent assay (19 mM, pH 7.2); adhesion molecule conjugate, 20 mM, pH 7.5.
5. Sigma Chemical Company Ltd., Poole, UK:

D-glucose; benzoic acid; o-dianisidine reagent; guar gum powder; hydrochloric acid; pancreatin (from porcine pancreatase); pepsin powder (from porcine gastric mucosa, 800-2500 U/mg); glucose oxidase diagnostic kit; anhydrous sodium acetate (≥ 99 %); calcium chloride (CaCl$_2$.6H$_2$O); Folin-Ciocalteu’s reagent; acetone (≥ 99.5 %); tannic acid; acarbose (> 95 %); poly-vinylpyrrolidone (PVPP); potassium ferricyanide; ferric chloride; ascorbic acid; trolox; 2,2-diphenyl-1-picrylhydrazyl (DPPH); ethylenediaminetetraacetic acid (EDTA); butylated hydroxytoluene (BHT); ferrozine ascorbate; H$_2$O$_2$; phosphate buffer; gallic acid; rutin; sodium nitrate; aluminium chloride; potato starch.

2.1.4 Buffers and solutions

1. Buffer concentrate kit (phosphate buffer) used in YSI 2357. It was made by dissolving the package contents into 500 ml of distilled water.

2. Buffer wash concentrate, 20 mM, pH 7.4, used in Evidence Investigator. 32 ml of buffer wash concentration was diluted with 968 ml of distilled water.

3. Enzyme preparation mixture (prepared immediately before use). Three g of pancreatin was added into each of six centrifuge tubes and suspended in 20 ml of water using a vortex-mixer. A magnetic stirring bar was added and the mixture was stirred for 10 min. The suspension was centrifuged at 1500 g for 10 min and 15 ml of the cloudy supernatant was removed from each tube and combined to give a total of 90 ml. Four ml of amyloglucosidase and 6 ml of invertase solutions were added, and the solutions mixed well by inversion.

4. Glucose standard solution. 5 g of glucose was weighed to the nearest 0.1 mg. The solution was then made up to 200 ml with sodium acetate buffer to give a concentration of 25 mg/ml glucose.

5. Pepsin Guar Gum solution. 1 g of pepsin powder was added to 200 ml of 0.05 mol/l hydrochloric acid and mixed with a magnetic stirring bar. Just before use, 1 g of guar gum was added and the solution was mixed well. This solution was prepared immediately prior to use.
6. Sodium acetate buffer, 0.1 mol/l, pH 5.2. 13.6 g of sodium acetate trihydrate was weighed and dissolved in 500 ml of water. Then, 250 ml of saturated benzoic acid solution was added and the solution was made up to 1 l with water after the solution was adjusted to pH 5.2 with 0.1 mol/l acetic acid. Four ml of 1 mol/l calcium chloride was added to 1 l of buffer to stabilize and activate the hydrolytic enzymes.

7. Sodium acetate, 0.25 mol/l. 34 g of sodium acetate trihydrate was weighed out and made up to 1 l with distilled water.

8. Sodium phosphate buffer, 200 mM, pH 6.6. 31.202 g of sodium phosphate was dissolved in 1 l of distilled water.

2.2 Methods

2.2.1 Procedures for the RAG and SAG measurements of reference standard foods

All procedures relating to the measurement of the carbohydrate fractions in the reference standard foods, with and without the presence of the cinnamon supplements (C. cassia, C. zeylanicum), were carried out using Englyst’s technique (Englyst et al., 2000). One reference standard food (cornflakes) was included in every batch of samples. The CV percentages of $G_{20}$ and $G_{120}$ (Table 2.1) were calculated.

Table 2.1 The mean, standard deviation and CVs for the reference food used to determine the inter-assay variation of RAG and SAG measurements.

<table>
<thead>
<tr>
<th>Reference food</th>
<th>Mean (g/100g)</th>
<th>SD</th>
<th>% CVs inter assay</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$G_{20}$</td>
<td>$G_{120}$</td>
<td>$G_{20}$</td>
</tr>
<tr>
<td>Cornflakes*</td>
<td>81.31</td>
<td>85.43</td>
<td>3.23</td>
</tr>
</tbody>
</table>

*Englyst food references were ordered from Englyst Carbohydrate Services Ltd., Southampton, UK.

2.2.1.1 The colorimetric approach

Fifty mg of guar gum powder was weighed into each of three 50 ml polypropylene centrifuge tubes with the addition of five glass balls. Into two of these tubes, 20 ml of glucose standard (25 mg/ml) (named as standard) was added, and into the third tube, 20 ml of acetate buffer (54.4 mg/ml) (named as blank) was added. Additional blanks and standards with a cinnamon supplement (a variety of doses, from 32 to 160 mg)
were also prepared. These tubes were treated exactly the same as the sample after adding sodium acetate solution.

Appropriate amounts of sample (0.8 g of cornflakes) with and without a cinnamon (from 32 to 160 mg) supplement were weighed into 50 ml polypropylene centrifuge tubes. Samples were analysed in duplicate. Ten ml of freshly prepared pepsin-guar gum solution was added into each sample tube, and then vortex-mixed before being placed in a water-bath at 37 °C for 30 min. After incubation, the samples were removed from the water-bath and five glass balls were added to each tube along with 10 ml of 0.25 mol/l sodium acetate. The tubes were shaken gently to disperse the contents.

The sample tubes, standards and blanks, with and without cinnamon, were placed in the water-bath at 37 °C to equilibrate for 10 min. After equilibration, the first sample was removed from the 37 °C water-bath, the cap removed and 5 ml of enzyme mixture added. The tube was then capped and the contents mixed gently by inversion. The tubes were then secured horizontally in the 37 °C shaking water-bath. The shaking action of the water-bath was initiated and this time called time zero of the incubation period. The shaking action was not interrupted until all of the G120 portions had been collected.

The addition of the enzyme mixture was repeated for the remainder of the sample tubes at 1 min intervals, and the tubes then placed in order into the shaking water-bath. After exactly 20 min of incubation, 0.5 ml of each sample was transferred into 20 ml of aqueous ethanol (66 %, v/v) and vortex-mixed to stop the hydrolysis reaction; this was the G20 portion. The sample tubes were then immediately returned to the shaking water-bath. After a further 100 min (total time 120 min), 0.5 ml of each sample was transferred from the sample tubes into 20 ml of aqueous ethanol (66 %, v/v) and vortex-mixed; this was the G120 portion. Then, the glucose released from the starch in the (G20, G120) samples, with or without the presence of cinnamon, was analysed colorimetrically, after appropriate treatment, using a spectrophotometer.

2.2.1.1.1 Spectrophotometric determination of glucose using glucose oxidase

Each of the G20 and G120 samples were centrifuged at 500 g for 5 min before the glucose levels were determined. In duplicate, 100 µl of sample (blank, samples and standards) was removed and pipetted into separate test tubes containing 2 ml of the
glucose oxidase regent, which had been prepared earlier according to manufacturer’s instructions. Then, all samples were vortex-mixed and incubated for 20 min at 37 °C. The absorbance of the standards and samples were measured at 510 nm against a reagent blank on a Uvikon 860 spectrophotometer.

The enzymatic colorimetric analysis was based on the production of glucose derived from the exposure of starch to digestive enzymes. The glucose produced was oxidised to gluconic acid and hydrogen peroxide by glucose oxidase. The hydrogen peroxide then reacts with \( \sigma \)-dianisidine in the presence of peroxidase to form a coloured product which is proportional to the original glucose concentration. The assay principle is demonstrated in the following reactions below:

\[
\begin{align*}
\text{Glucose} + \text{H}_2\text{O} + \text{O}_2 & \xrightarrow{\text{Glucose oxidase}} \text{Glucose oxidase} \xrightarrow{\text{Gluconic acid} + \text{H}_2\text{O}} \\
\text{H}_2\text{O}_2 + \text{Reduced Dianisidine} & \xrightarrow{\text{Peroxidase}} \text{Oxidized } \sigma\text{-dianisidine (colour)}
\end{align*}
\]

The glucose concentration (gram per 100 g of sample) is given by following equation:

\[
\text{Glucose (%)} = \frac{A(t) \times V \times C \times 100}{A(s) \times W}
\]

\( A(t) \) is the absorbance of the test solution; \( V \) is the total volume of the test solution\(^1\) (ml), \( C \) is the concentration\(^2\) (mg/ml) of the standard used; \( A(s) \) is the absorbance of the standard used; \( W \) is the weight (mg) of the sample taken for analysis.

The values for RAG and SAG were calculated from the \( G_{20} \) and \( G_{120} \) values and obtained as follows:

\[
\begin{align*}
\text{RAG (g)} &= G_{20} \\
\text{SAG (g)} &= G_{20} - G_{120}
\end{align*}
\]

\(^{1} V = 20 \text{ ml}; ^{2} C = 25 \text{ mg} \) (Englyst’s et al., 2000).

### 2.2.2 Preparation of the cinnamon extracts

Polyphenols can be extracted a number of different ways; proanthocyanidins and the procyanidin fraction can be extracted using pure or aqueous methanol, pure or aqueous ethanol, or acetone. For the extraction of procyanidins, acetone has been shown to possess the highest extraction efficiency followed by ethanol and methanol (Lazarus et al., 2001). Therefore, acetone solvent at a concentration of 75 % (\( v/v \)) was
used. In addition for comparison, deionised water was used to extract the cinnamon samples.

Cinnamon samples were extracted according to Mathew and Abraham (2006) with a slight modification in term of duration of stirring and temperature. These extracts were used to determine the total polyphenol, tannin and proanthocyanidin content.

Deionised was added to each cinnamon supplement sample (1.5 g) and continuously stirred at 4 °C for 7 hours. The extraction process was repeated (three times) until the water extract became colorless. This was called cinnamon water extract. The same procedure was followed using 75 % acetone (v/v). This was called cinnamon 75 % acetone extract. The total solvent volume was 300 ml in each case.

2.2.3 Measurement of the total phenolic content

The total phenol content of the extracts was determined using the Folin Ciocalteu method described by Lv et al. (2012), with a slight modification. Briefly, 40 µl of each cinnamon extract was added to 200 µl of Folin-Ciocalteu reagent. Following a 5 min incubation period, 600 µl of sodium carbonate (20 % (w/v)) was added and the samples left to stand in darkness for 2 h. The absorbance of the samples was read at 750 nm against a blank. The standard curve was determined using gallic acid (0, 50, 100, 200, 300, 400, 500, 600, and 700 µg/ml) (w/v). The results were expressed as gallic acid equivalents.

2.2.4 Measurement of hydrolysable tannins

This method is based on the total phenol content present in the cinnamon extracts, which were prepared above (section 2.2.3). An insoluble matrix compound, polyvinylpolypyrrolidone (PVPP), which binds with cinnamon tannin phenolic compounds, enabled measurement of the cinnamon tannins. The tannin content was determined according to Makkar et al. (2000). The method is based on the principle that 100 mg of PVPP is sufficient to bind 2 mg of total phenols. The method required 1 ml of distilled water and 1 ml of each cinnamon extract for each 100 mg of PVPP. The appropriate amount of PVPP, which depended on the total phenols of each cinnamon sample, distilled water and cinnamon extract were vortex mixed. The mixture was incubated in a cool environment (4 °C) for 15 minutes. The mixture was vortex-mixed again and centrifuged at 3000 g for 10 min. The supernatant was collected to determine the total
phenolic content after centrifugation (section 2.2.3). The total tannin content was calculated by subtracting the value of total phenolic compounds without PVPP from the total phenolic compounds with PVPP, equivalent to tannic acid.

2.2.5 Extraction of the cinnamon samples for HPLC and LC-MS

The cinnamon samples that were extracted for the HPLC and LC-MS analyser were the pure cinnamon samples of *C. cassia* and *C. zeylanicum*. The Plant for Human Health Institution in North Carolina State University, United States, analysed the *C. cassia* and *C. zeylanicum*, and HPLC and LC-MS were used to determine the polyphenol composition in the cinnamon samples. Moreover, they extracted the cinnamon samples (2.2.5.1), and then determined the total polyphenol (2.2.5.2) and condensed tannin (proanthocyanidins) (2.2.6) contents.

2.2.5.1 Extraction of pure cinnamon supplements

*C. cassia* and *C. zeylanicum* powders (0.500 mg) were extracted with 8 ml acetone 75 % (v/v) by sonication at 55 °C for 5 min. The mixture was then centrifuged for 20 min at 1792 g and the supernatant transferred to 25 ml volumetric flasks. Extraction of the pellet was repeated twice more and the combined extracts brought to a final volume of 25 ml. One ml of the cinnamon extracts was filtered using a 0.2 mm PTFE syringe filter and placed in HPLC amber vials (Fisher Scientific, Pittsburg, PA, USA), before phytochemical analyses using HPLC and LC-MS.

2.2.5.2 Measurement of the total phenols

The total phenol content of the extracts was determined using the Folin Ciocalteu method (2.2.3).

2.2.6 Measurement of the condensed tannins (proanthocyanidins)

The Plant for Human Health Institution in North Carolina State University, United States, determined the total proanthocyanidin (PAC) concentrations. The PAC was determined colorimetrically using the DMAC method (2.2.7) in a 96-well plate according to Prior et al. (2010). A series of dilutions of standard procyanidin A2 dimer were prepared and ranged from 1-100 µg/ml. Blank, standards and diluted samples were analysed in triplicate. The plate reader protocol was set to read the absorbance at 640 nm for each well in every plate. The concentration of PAC in the solution was expressed as mg/l procyanidin A2 equivalents.
2.2.7 The DMAC method

DMAC (4-dimethylaminocinnamaldehyde) is a colorimetric method which was used to measure the total of proanthocyanidins. The procyanidin A2 dimer (Figure 2.1) was used as standard (Prior et al., 2010).

![Figure 2.1: Procyanidin A2 dimer (Prior et al., 2010).](image)

2.2.7.1 Procyanidin A2 standard preparation

Five mg of the standard procyanidin A2 was weighed and quantitatively transferred to a 50 ml volumetric flask. Ethanol (91 %, v/v) was added to give a final concentration of 0.100 mg/ml. Aliquots were placed in 1.5 ml HPLC sample vials and stored at −80 °C. These standards remained stable for at least 6 months.

2.2.7.2 Procyanidin A2 control preparation

A procyanidin A2 control was prepared by transferring 1 ml of the 0.100 mg/ml procyanidin A2 solution into a 1.5 ml conical tube. To this, 250 μl of ethanol (91 %, v/v) was added and vortexed to give 0.80 mg/ml procyanidin A2 as a quality control sample.

2.2.7.3 96-Well plate layout

The absorbance at 640 nm was read for each well in the plate every minute for 30 minutes. The plate included blanks, standards, controls, and cinnamon extracts at serial dilutions of 1-, 2-, 4-, 8-, 16-, and 32-fold.
2.2.7.4 Data analysis

The maximum absorbance readings were used for calculation; this occurred at 20 min. PAC concentrations were calculated by using a regression equation:

\[ Y = a + bX \]

\( Y \) is procyanidin A2 concentration in \( \mu g \); \( a \) is the maximum absorbance; \( b \) is the blank and \( X \) is the maximum absorbance minus the blank (\( X \)).

Concentrations of sample extracts were calculated as total:

\[ \text{PAC} = \frac{C \times D \times V}{1000 \times S} \]

PAC values are in mg/g; \( C \) is the concentration of PACs in a sample extract in g/l; \( D \) is the dilution factor; \( V \) is the extraction volume in ml and \( S \) is the sample size, in g. Data were expressed as mg of procyanidin A2 equivalents per g or per 300 ml of sample.

2.2.8 HPLC analysis

HPLC analyses were conducted using an Agilent 1200 HPLC with fluorescence detection (FLD) and photodiode array detection (PAD). The Wallace and Giusti (2010) method was used for separating the different proanthocyanidins. A Develosil Diol column was used (250 mm x 4.6 mm (internal diameter) x 5 \( \mu m \), Phenomenex, Torrance, CA, USA). PACs were identified by comparison with the available standards. Quantification of the proanthocyanidins was calculated using peak areas and a calibration curve for procyanidin A2, and amounts were expressed as procyanidin A2 equivalents. The mobile phase was 2 \% (v/v) acetic acid in distilled water (solvent A) and 0.5 \% (v/v) acetic acid in 50 \% acetonitrile in distilled water (solvent B). The flow rate was 1 ml/min with a step gradient of 10 \%, 55 \%, 100 \% and 10 \% of solvent B at 0, 10, 13, 15, 20, 50, 54 and 60 min respectively. Peak areas recorded at 280 nm were quantified using a calibration curve obtained using a procyanidin A2 standard (Grace et al., 2009, 2013).

2.2.9 LC-MS analysis

LC-MS consisted of a LC-MS-IT-TOF instrument (Shimadzu, Tokyo, Japan) and a HPLC system (SIL-20A HT auto-sampler, LC-20AD pump system, SDP-M20A photodiode array detector). The LC separation was performed using a C18 reverse-phase column (Shim-pack XR-ODS column, 50 mm x 3.0 mm i.d. x 2.2 \( \mu m \) particle size,
Shimadzu Scientific Inst., Columbia, MD, USA). The mobile phase consisted of 2 % (v/v) acetic acid in distilled H₂O (solvent A) and 50 % acetonitrile in distilled H₂O (solvent B). The flow rate was set at 0.35 ml/min with a step gradient of solvent B of 6-25 % (18 min), 25-60 % (2 min), 60 % (5 min), 60-6 % (2 min), 6 % (3 min). The column was re-equilibrated for 5 min at initial conditions (6 %, solvent B). The heat block and curved desolvation line (CDL) were maintained at 200 °C. Nitrogen gas was used as a nebulizer and drying gas with the flow rate set at 1.5 l/min. The ESI source voltage was set at 4.5 kV and the detector was set at 1.5 V. The instrument was calibrated to <5 ppm error in mass accuracy with an external standard of sodium TFA solution. Ionization was performed using a conventional ESI source in negative ionization mode. Data was acquired at m/z in the 150-2000 range (Grace et al., 2009, 2013).

2.2.10 Determination of the antioxidant activity of the cinnamon extracts

Determination of the antioxidant activity was carried out with pure cinnamon powder supplement extracts of cinnamon cassia (C. cassia) and cinnamon zeylanicum (C. zeylanicum).

2.2.10.1 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The total radical scavenging capacity of cinnamon extracts was determined using an adaptation of the method described by Mau et al. (2004). This method was used to measure the antioxidant activity of the compounds in the cinnamon extracts. The principle of the assay is based on the fact that when antioxidant compounds donate H⁺ to the DPPH molecule, the DPPH is reduced forming a colourless compound (Brand-Williams et al., 1995; Molyneux, 2004). Thus, the greater the hydrogen donating property of a compound, the greater the rate of decolourisation. Trolox and BHT (0.1 mg/ml) were used as controls. Trolox was dissolved in deionised water whereas DPPH was dissolved in aqueous 75 % acetone (v/v). Different concentrations of the cinnamon extracts were prepared (0.05, 0.100, 0.150 mg/ml) for all the cinnamon extracts (water-based C. cassia extract, water-based C. zeylanicum extract, acetone C. cassia extract and acetone C. zeylanicum extract). Two ml of the samples or control (Trolox and BHT) was added to 0.5 ml of 0.2 mM DPPH. The samples were left to stand in the dark for 30 min at room temperature and the absorbance was read in the spectrometer at 517 nm. All analyses were performed in triplicate with the relevant blank for each
concentration. The percentage of DPPH radical scavenging activity was calculated using the following equation:

\[
\% \text{ DPPH} = \left(\frac{\text{control absorbance} - \text{test sample}}{\text{control absorbance}}\right) \times 100
\]

### 2.2.10.2 Scavenging of hydrogen peroxide (H\textsubscript{2}O\textsubscript{2})

The hydrogen peroxide scavenging activity of cinnamon sample extracts was determined using the method of Gülçin et al. (2007). Different concentrations of cinnamon extracts (0.05, 0.100, 0.150 mg/ml) were used. H\textsubscript{2}O\textsubscript{2} (40 mM) was prepared in phosphate buffer at pH 7.4 and 0.6 ml added to 1 ml of each of the cinnamon extracts. The samples were incubated for 10 min at room temperature, then the absorbance of the samples and controls (BHT and trolox (0.1 mg/ml)) were read at 230 nm. All tests and analyses were performed in triplicate with a relevant blank run for each concentration. The percentage inhibition activity was calculated using the following equation:

\[
\text{Inhibition activity (\%) = } \left(\frac{\text{control absorbance} - \text{test sample}}{\text{control absorbance}}\right) \times 100
\]

### 2.2.10.3 Metal chelating activity

The chelation of ferrous ions by the different cinnamon extracts and standards was estimated using a slight modification to the method of Dinis et al. (1994). Different concentrations of cinnamon extracts were prepared (0.05, 0.100, 0.150 mg/ml). 0.500 ml from the samples and 1.5 ml of deionised water were added to 50 µl of 1 mM FeCl\textsubscript{2} and vortexed, then 100 µl of 1 mM ferrozine was added to the mixture, and they were incubated for 10 min at room temperature. The absorbance of the solution was measured spectrophotometrically at 562 nm. All tests and analyses were performed in triplicate with a relevant blank for each concentration. EDTA (0.1 mg/ml) was used as control. The percentage of inhibition of ferrozine Fe\textsuperscript{2+} complex formation was calculated using the formula given below:

\[
\% \text{ inhibition} = \left(\frac{\text{control absorbance} - \text{test sample}}{\text{control absorbance}}\right) \times 100
\]

### 2.2.10.4 Reducing power

The reducing power was determined according to the method of Oyaizu (1986). This method is based on determining the ability of the tested samples to reduce Fe\textsuperscript{3+} (CN)\textsubscript{6} to Fe\textsuperscript{2+}(CN)\textsubscript{6}, which is a blue-coloured complex. Different cinnamon extract
concentrations were prepared (0.05, 0.100, 0.150 mg/ml) for analysis. 2.5 ml of each extract was mixed with 2.5 ml of 200 mM phosphate buffer (pH 6.6) and 2.5 ml of 1 % potassium ferricyanide (w/v). The mixture was incubated at 50 °C for 20 min in a water bath. Then, 2.5 ml of 10 % of trichloroacetic acid was added followed by mixing and centrifugation at 1000 g for 10 min. The upper layer (3 ml) was mixed with 3 ml of deionised water and 1 ml of 1 % ferric chloride (w/v), and allowed to stand for 10 min in room temperature. The absorbance was read spectrophotometrically at 700 nm. A higher absorbance denotes a higher reducing power. All tests and analyses were performed in triplicate with a relevant blank for each concentration.

2.2.11 The clinical studies

Acute and long-term human studies were run in this project; both received ethical approval from the University of Surrey Ethics Committee (EC/2011/145/FHMS and EC/2013/70/FHMS respectively).

2.2.11.1 Participant recruitment

All subjects for the clinical studies were recruited from the University of Surrey staff population, as well as around Surrey, by the distribution of both e-mails and posters. For the long-term human studies, in addition to distributing the poster by e-mail, the recruitment poster for the study was placed in several churches, GP surgeries, hospitals and bus stops of Woking, Guildford and Kingston.

2.2.11.2 Screening

All participants were screened prior to each study in order to check that they met the specific study inclusion criteria. Screening for the acute study required each participant to attend the CIU for one morning and fill in the health and lifestyle questionnaire. For the long-term study, each participant attended one morning having fasted overnight, and the procedures described below were carried out.

2.2.11.2.1 Health and lifestyle questionnaire

The questionnaire for acute and long-term human studies included questions regarding the participant’s personal details, general health and lifestyle aspects. Participants with current or previous medical conditions, for instance, diabetes, were excluded.
2.2.11.2.2 Blood glucose testing

Fasting blood glucose levels were analysed from blood samples obtained using the finger prick method for long term study. The blood samples were checked using Freestyle Optium. Participants with concentrations outside of the normal range of fasting blood glucose (>6 mmol/l) were excluded from the studies. This applied to the long-term human studies during screening.

2.2.11.2.3 Following and monitoring the participants

Each participant received a reminder, an email and text message, which included instructions to follow, one week prior to their visit. A further reminder was sent three days prior the study day and a final reminder sent one day prior the visit.

2.2.11.2.4 Anthropometric measurements

Height, weight, body mass index (BMI) and blood pressure were taken at the baseline for each study. All of these measurements were taken by the same investigator within a study in order to reduce inter-operator variation.

2.2.11.2.4.1 Height

A standard stadiometer (Seca Ltd., Birmingham, UK) was used to measure the height of the participants in centimetres. The participants were asked to remove their shoes, and then they stood straight with their feet together and their back against the stadiometer.

2.2.11.2.4.2 Weight and body mass index

Weight and BMI was determined using Tanita scales (Tanita TBF-300, Tanita UK Ltd., Middx, UK) with shoes and socks removed.

2.2.11.2.4.3 Waist and hip circumference

Waist and hip circumferences were measured to the nearest 0.1 cm using an Idass tape measure (Idass Ltd., Kettering, Northamptonshire).

2.2.11.2.4.4 pQCT scanner

A pQCT scanner was used to determine the bone density of the participants. It produced radiation less than 1/10 of a normal chest X-ray. The procedure involved the
participant sitting on a seat and placing their forearm into the scanning machine. The X-ray beam then moved across the forearm (Appendix 1).

**2.2.11.2.4.5 Blood pressure**

Blood pressure was measured three times using an automatic blood pressure cuff (Omron MX3 Plus, Omron Healthcare Europe, and UK) with the subjects sitting at rest. An average of the three readings was then taken.

**2.2.11.3 Capsule filling procedure**

The filling process followed USA Food and Drug Administration (FDA) rules. Each cinnamon (*C. cassia*) supplement capsule was opened and the cinnamon powder collected in a glass container. Then, the manual capsule filling machine CN-400/CN-400CL was used to produce new cinnamon or placebo dark green capsules, which were identical in size and weight. These capsules were used for long term study.

The base plate, (cap sheet, bottom sheet) was placed on a clean and dry surface. The filling tray, (encapsulation sheet) was placed and secured on the top of the base plate. The long part of the capsules was poured into the filling tray, and then shaken gently to fill all of the holes with capsules. Then the filling tray was removed, and any excess long part of the capsules were removed. The filling tray (encapsulation sheet) was placed and secured on the top of the upper plate. The short part of the capsules were poured into filling tray, which were placed on the upper plate, and shaken gently to fill all of the holes with capsules. Any excess short part of the capsules were removed, and then the filling tray was removed. The powder keeper was secured on the top of the base plate. The cinnamon powder or cornflower was then added to fill the capsules, which were on the base plate. The spreader was used to move the cinnamon powder back and forth to fill the capsules, and the tamping tool was used to compress the fillings, after which the previous procedure was repeated. The middle (joint) plate was placed on the top of the upper plate and pushed firmly, to form one unit. This part was then turned over and placed on the top of the base plate and pushed gentle, and the upper plate was removed. Finally, the middle (joined) plate, which has the joined capsules, was removed carefully and the capsules were collected in clean container. A specific number of capsules were added to the medical tablet bottles and one bottle was handed to each participant on visit 1 and 2 (Appendix 2).
The intra CV% of capsules was 3.27 and inter of capsules CV% was 6.28. This shows they had good reproducibility between the capsules.

2.2.11.4 Biochemical analysis

2.2.11.4.1 Blood sample collection

Capillary and venous blood sampling methods were applied. In the acute study, capillary finger prick blood samples were collected into 300 µl microvette tubes coated with fluoride oxalate, and were immediately centrifuged at 2000 g for 10 min at 4 °C. The centrifuged plasma was transferred into separate 300 µl plastic plain microvette tubes which were then kept in the freezer at -20 °C until analysis (within 3 weeks).

In the long-term study, the venous blood samples were collected into vacutainer tubes containing the following anticoagulants: EDTA for determination CHO, TAG, LDL, HDL, NEFA; Fluoride oxalate for determination of the plasma glucose; and Lithium heparin for plasma insulin. Serum tubes, which did not contain any additive were used to determine cytokines (IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, EGF, IFN-γ, MCP-1, TNF-α, VEGF), adhesion molecules (VCAM, ICAM, E-selectin, P-selectin, L-selectin) and C reactive protein (CRP). The blood was immediately centrifuged at 2000 g for 10 min at 4 °C. The serum samples were left for 25 min to clot and then centrifuged at 2000 g for 10 min at 4 °C. Aliquots of plasma and serum were dispensed into appropriately labelled microcentrifuge tubes (1.5 ml) and stored at -80 °C until analysis.

2.2.11.4.2 Analyses of plasma glucose

The plasma glucose concentration in the acute study was determined using YSI 2300 STAT plus analyser, Yellow Springs, UK. The principle for the colorimetric YSI analyser method is based on an enzyme for glucose being placed between the membrane layers, which are polycarbonate and cellulose acetate. In the present of hydrogen peroxide, glucose is oxidised, and the oxidised glucose enters the enzyme layer. The hydrogen peroxide produced passes through the cellulose acetate membrane into a platinum electrode where the hydrogen peroxide is oxidised.

\[
\begin{align*}
\text{D-glucose} + \text{O}_2 & \underset{\text{Glucose oxidase}}{\rightarrow} \text{D-glucono-\alpha-lactone} + \text{H}_2\text{O}_2 \\
\text{H}_2\text{O}_2 & \underset{\text{Platinum anode}}{\rightarrow} 2\text{H}^+ + \text{O}_2 + 2e^{-}
\end{align*}
\]
Twenty-four samples were analysed in each run along with three quality controls. The mean and standard deviation of the quality controls were used to determine the intra- and inter-assay coefficient of variation (Table 2.2). The results show there were very low levels of intra and inter assay variation, suggesting this assay is reproducible and the results are representative of the samples.

Table 2.2 The three quality controls (QCs) that used within automatic analyser (YSI2300 STAT).

<table>
<thead>
<tr>
<th>QCs</th>
<th>Mean mmol/l</th>
<th>SD</th>
<th>Intra assay % CV</th>
<th>Mean mmol/l</th>
<th>SD</th>
<th>Inter assay % CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>4.85</td>
<td>0.05</td>
<td>1.16</td>
<td>4.93</td>
<td>0.11</td>
<td>2.17</td>
</tr>
<tr>
<td>Medium</td>
<td>6.84</td>
<td>0.06</td>
<td>0.82</td>
<td>6.73</td>
<td>0.13</td>
<td>1.90</td>
</tr>
<tr>
<td>High</td>
<td>24.35</td>
<td>0.07</td>
<td>0.29</td>
<td>24.60</td>
<td>0.23</td>
<td>0.93</td>
</tr>
</tbody>
</table>

In the long-term study, the ILab 650 method (Instrumentation Laboratory, UK) was used to determine the fasting plasma glucose. The ILab 650 method principle is based on enzymatic oxidation of glucose in the presence of glucose oxidase (GOD). The resultant hydrogen peroxide reacts with phenol and 4-aminophenzone under the catalysis of peroxidase (POD) to produce a red–violet quinoneimine dye. The absorbance of the dye was measured at 510 nm.

\[
\begin{align*}
\text{D-glucose} + \text{O}_2 + \text{H}_2\text{O} & \xrightarrow{\text{GOD}} \text{Gluconic acid} + \text{H}_2\text{O}_2 \\
2\text{H}_2\text{O}_2 + 4\text{-aminophenzone} + \text{phenol} & \xrightarrow{\text{POD}} \text{Quinineimine} + 4\text{H}_2\text{O}
\end{align*}
\]

Quality controls (QCs) were measured at the beginning and end of each run.

Intra-assay precision gave CVs of 0.67 % for level 1 (low) QC, 0.49 % for level 2 (high) QC. Inter-assay precision gave CVs of 0.77 % and 0.66 % for both level 1 and level 2 QCs, respectively, which again shows the accuracy and reproducibility of this method.

**2.2.11.4.3 Analyses of plasma cholesterol**

The enzymatic colorimetric method ILab 650 (Instrumentation Laboratory, UK) was used to determine total cholesterol. Cholesterol esters were hydrolysed to free cholesterol and free fatty acids by pancreatic cholesterol esterase. The liberated
cholesterol and any free cholesterol present in the plasma were both oxidized by cholesterol oxidase. The assay principle of the reactions is shown below:

\[ \text{Cholesteryl ester} + \text{H}_2\text{O} \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol} + \text{Fatty acids} \]

\[ \text{Cholesterol} + \text{O}_2 \xrightarrow{\text{Cholesterol oxidase}} \text{Cholest-4-en-3-one} + \text{H}_2\text{O}_2 \]

\[ 2\text{H}_2\text{O}_2 + 4\text{-aminophenazone} + \text{phenol} \xrightarrow{\text{Peroxidase}} \text{4-(p-benzoquinonemonoimino)-phenazone} + 4\text{H}_2\text{O} \]

The absorbance of the quinoneimine pigment was measured at 510 nm. The intra-assay precision gave CVs for level 1 (low) and level 2 (high) QC of 1.02 % and 1.61 % respectively. Inter-assay precision gave CVs for level 1 (low) and level 2 (high) QCs of 1.90 % and 2.17 % respectively. These results show there was very low inter and intra assay variability, meaning the data produced was accurate.

### 2.2.11.4.4 Analysis of plasma TAG

The plasma TAG concentrations were measured using the automated colorimetric method (ILab 650 analyser, Instrumentation Laboratory, UK). TAG was hydrolysed to glycerol and free fatty acids using lipoprotein lipase, then the glycerol kinase present within the assay kit acts upon glycerol and converts it into glycerol-3-phosphate. This is oxidised by glycerophosphate oxidase to dihydroxyacetone phosphate, and the concentration of the quinoneimine dye generated in the reaction is proportional to the concentration of TAG in the sample. The absorbance was measured at 510 nm.

\[ \text{Triacylglycerides} + 3\text{H}_2\text{O} \xrightarrow{\text{Lipoprotein Lipase}} \text{Glycerol} + \text{Fatty acids} \]

\[ \text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerol Kinase}} \text{Glycerol-3-phosphate} + \text{ADP} \]

\[ \text{Glycerol-3-phosphate} + \text{O}_2 \xrightarrow{\text{GPO}} \text{Dihydroxyacetone-phosphate} + \text{H}_2\text{O}_2 \]

\[ \text{H}_2\text{O}_2 + 4\text{-chlorophenol} + 4\text{-aminoantipyrine} \xrightarrow{\text{POD}} \text{Quinoneimine dye} + \text{H}_2\text{O} \]

Where POD = peroxidase; GPO = glycerol-3-phosphate oxidase; ADP = Adenosine-5'-diphosphate.

The within assay precision gave CVs for level 1 (low) and level 2 (high) QC of 1.17 % and 1.60 % respectively, while the between assay precision gave CVs of 1.06 % and 1.74 % for the low and high quality control, respectively. These results show the
variability was Lew for this assay, suggesting there was good reproducibility within the standards.

### 2.2.11.4.5 Analyses of plasma HDL

The assay principle is based on the following reactions:

\[
\text{Cholesterol esters} + \text{H}_2\text{O} \xrightarrow{\text{Cholesterol esterase}} \text{Unesterified cholesterol} + \text{fatty acids}
\]

\[
\text{Unesterified cholesterol} + \text{O}_2 \xrightarrow{\text{Cholesterol oxidase}} \text{Cholestone} + \text{H}_2\text{O}_2
\]

\[
2\text{H}_2\text{O}_2 + 5\text{-Aminoantipyrine} + \text{N-ethyl-N-(3-methylphenyl)-N'ssuccinylethylene- diamine}
+ \text{H}_2\text{O} + \text{H}^+ \xrightarrow{\text{peroxidase}} \text{Qunoneimine dye} + \text{H}_2\text{O}
\]

When measured at 600 nm, the intensity of the quinoneimine dye product was directly proportional to the cholesterol concentration. Within assay precision gave CVs for level 1 (low) and level 2 (high) QC of 0.98 % and 0.42 %, respectively. The inter-assay precision gave CVs of 2.07 % and 0.76 %, respectively.

VLDL and LDL were calculated from the Friedewald (1972) Formula:

\[
\text{LDL} = \text{T. cholesterol} - \left( \text{HDL} - \text{TG} \right)/2.17 \text{ (mmol/l)}
\]

### 2.2.11.4.6 Analyses of plasma NEFA

Automated colorimetric method (ILab 650 analyser) was used to determine NEFA values. The enzymatic reaction of acyl CoA synthetase with NEFA, followed by the reaction of acyl CoA with acyl CoA oxidase, produces hydrogen peroxide; the hydrogen peroxide reacts with 4-aminoantipyrine which results in formation of a purple dye that absorbs at a wavelength of 550 nm. The intensity of the colour is directly proportional to the NEFA concentration.

\[
\text{NEFA} + \text{ATP} + \text{CoA} \xrightarrow{\text{synthetase}} \text{Acyl CoA} + \text{AMP} + \text{PPi}
\]

\[
\text{Acyl CoA} + \text{O}_2 \xrightarrow{\text{acyl CoA oxidase}} 2,3\text{-trans-Enoyl- CoA} + \text{H}_2\text{O}_2
\]

\[
2\text{H}_2\text{O}_2 + \text{TOOS} + 4\text{-aminoantipyrine} \xrightarrow{\text{peroxidase}} \text{Qunoneimine dye (purple adduct)}
+ 4\text{H}_2\text{O}
\]

Where TOOS = \text{N-ethyl-N-(2-hydroxy-3-sulphopropyl) m-toluidine. Two QCs were employed and the CVs for the low and high were found to be 3.29 % and 1.74 %,
respectively. The between assay precision gave CVs of 3.33 % and 1.33 % for the low and high QCs, respectively.

2.2.11.4.7 Analyses of serum C reactive protein (CRP)

The automated colorimetric method (ILab 650 analyser) was used to determine the CRP levels. The immunoturbidimetric method is based on highly specific binding between an antigen and an antibody. Latex particles reacted with an antibody specific to human CRP aggregate in the presence of CRP in the sample to produce immune complexes, which increased in turbidity. These immune complexes cause an increase in light emission which is related to the concentration of CRP in the serum. The absorption of light scattering was measured at 570 nm. The CRP concentration was determined from a calibration curve developed from CRP standards of known concentration. Intra-assay precision gave CVs of 5.39 % for the level 1 (low) QC and 8.10% for level 2 (high) QC. Inter-assay precision gave CVs of 8.65 % and 7.85 % for both level 1 (low) and level 2 (high) QCs, respectively.

2.2.11.4.8 Analyses of plasma insulin

Plasma insulin concentrations were determined using a radioimmunoassay. The Millipore Human Insulin, which utilized 125I-labeled human insulin and human insulin anti-serum to determine the level of insulin in the plasma, was used. This method requires two days of setting up; plasma samples were defrosted at room temperature, different standard concentrations of insulin were prepared (200, 100, 50, 25, 12.5, 6.25, 3.125µU/ml), 300 µl of assay buffer was added to non-specific binding (NSB) tubes and 200 µl to the reference (BO) tubes and then 100 µl of each standard and Quality Control were added. 100 µl of buffer was added to all sample tubes, followed by the addition of 100 µl of each plasma sample. 125I-Insulin label was prepared (27 ml of ready hydration solution was added to 125I-Insulin, mixed gently and left at room temperature for 30 minutes), and 100 µl was added to all the tubes, then 100 µl of human insulin antibody was then added to all tubes except the total count and NSB. Tubes were vortex mixed, covered and incubated overnight (21 hours) at room temperature (23 °C). On the second day, 1 ml of cooled precipitating reagent (4 °C) was added to all tubes except total count tubes and vortex mixed. The tubes were incubated for 20 min at 4 °C and then all of the tubes were centrifuged, except the total count tubes, at 2500 x g for 20 min. The supernatants from all of the tubes were
aspirated under a vacuum and the pellet containing the antibody bound insulin was counted on a gamma counter for 1 minute per sample (Wizard 1470, Wallac International, and Finland). Each sample was counted against the standard curve in order to ascertain the concentration of insulin in each sample.

Low and high QC plasma samples were included at the beginning and the end of each assay. The intra-assay precision CV% for high and low were 1.20 % and 2.27 %, respectively. The inter-assay precision CV% for high and low were 3.50 % and 9.54 %, respectively.

2.2.11.4.8.1 Calculation of the homeostasis assessment model (HOMA)

The Homeostasis Model Assessment (HOMA1) calculator was used to determine the beta cell function (%B) and insulin resistance (IR). This calculator was originally produced by Mattewa et al. (1985), and was calculated as described by Wallace et al. (2004) using the following formula:

\[
\text{HOMA1 (IR)} = \frac{\text{FPI} \times \text{FPG}}{22.5}
\]

\[
\text{HOMA1 (%B)} = \frac{20 \times \text{FPI}}{\text{FPG} - 3.5}
\]

Levy et al. (1998) updated HOMA1 to HOMA2. In 2004, the HOMA2 calculator in computer was released, which is available from www.dtu.ox.ac.uk/homacalculator/. This model can be used to determine insulin resistance (IR), insulin sensitivity (%S) and β-cell function (%B) (Wallace et al., 2004). This model calculated required fasting glucose and insulin value. There is no approximation formula for HOMA-2. The HOMA2 calculator was used in this study to estimated beta cell function (%B) and insulin resistance (IR) and insulin sensitivity (%S).

2.2.11.4.9 Evidence Investigator

Biochip array technology in combination with the Evidence Investigator instrument (Randox Laboratories Ltd., Crumlin, UK) was used to determine multiple cytokines from a single sample in real time. The Investigator system consists of: a Biochip carrier, which includes a solid phase of 9 biochip (9 mm²); nine multi-analyte calibration levels; handheld barcode scanner to input the details of the calibration and cytokines automatically into the system database; three quality controls; a thermo-shaker to provide fluid flow and mixing within the wells across the biochip surface; and a charge
coupled device (CCD) camera to recorded the light emitted from cytokines which bind
to the discrete test regions (DTRs). The Evidence assay kit allows up to 54 samples to
be processed within the Evidence analyser (Appendix 3).

2.2.11.4.9.1 Principle of biochip array technology

The protein sandwich immunoassay method was used to detect the serum cytokines
and adhesion molecules. The protein arrays constructed on the biochip surface allow
the cytokines and adhesion molecules (ligand, analytes) to bind to their primary
antibody. Horseradish peroxidase (HRP) labelled secondary antibody, present as a
conjugate, detected the analytes bound to the biochip surface. The signal reagent
EV805 produced a light emission (chemiluminescent) reaction on the surface of the
biochip, and was measured by a super-cooled CCD camera. The CCD camera
simultaneously recorded the light emission from all of the biochips. The Evidence
software was used to calculate the cytokine and adhesion molecule concentrations
from the calibration curves (Appendix 3).

2.2.11.4.9.2 Determination of high sensitivity cytokines

The Evidence Investigator (Randox Laboratories Ltd., Crumlin, UK) was used to
determine the 12 cytokines with high sensitivity (IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-
10, VEGF, IFN-γ, MCP-1, TNF-α, and EGF) simultaneously from a single sample. The
functional sensitivity of cytokines was as follows: IL-1α: 0.19 pg/ml; IL-1β: 0.26 pg/ml;
IL-2: 2.97 pg/ml; IL-4: 2.12 pg/ml; IL-6: 0.12 pg/ml; IL-8: 0.36 pg/ml; IL-10: 0.37 pg/ml;
VEGF: 3.24 pg/ml; IFN-γ: 0.44 pg/ml; MCP-1: 3.53 pg/ml; TNF-α: 0.59 pg/ml; EGF:
1.04 pg/ml (Molloy et al., 2005; FitzGerald et al., 2008; Fabre et al., 2008).

2.2.11.4.9.2.1 Cytokine high sensitivity approach

200 µl of assay diluent was added to each biochip well. A total of 100 µl of undiluted
serum samples, calibration and quality controls were added to the biochip well and
tapped gently. The samples were incubated in the thermo-shaker for one hour at 37
°C and 15 g. Then, the biochip samples were incubated for 19 hours at 4 °C. Wash
buffer was used to wash the biochip well four times, then 300 µl of the conjugate was
added into each biochip well and taped gently. After this, the samples were incubated
in the thermo-shaker for one hour at 37 °C and 15 g. 250 µl of signal reagent EV805
(Luminol and Peroxide, 1:1) was added to each biochip well, then wash buffer was
used to wash the biochip wells twice. The Biochip wells were filled with wash buffer and then uploaded into the Evidence analyser to take an image for each biochip well. Subsequently, the cytokines were quantified automatically using generated calibration curves (Evidence Investigator Software) (Molloy et al., 2005; FitzGerald et al., 2008; Fabre et al., 2005).

2.2.11.4.9.3 Determination of the adhesion molecule levels
VCAM, ICAM, E-selectin, P-selectin, L-selectin were determined using the Evidence Investigator (Randox Laboratories Ltd., Crumlin, UK) simultaneously from a single serum sample. The functional sensitivity for the adhesion molecules was as follows: VCAM: 4.1 ng/ml; ICAM: 1.7 ng/ml; E-selectin: 0.1 ng/ml; P-selectin: 1.9 ng/ml; L-selectin: 3.2 ng/ml (Molloy et al., 2005; FitzGerald et al., 2008; Fabre et al., 2005).

2.2.11.4.9.3.1 Determination of the adhesion molecule approach
Serum samples were diluted (1:10); 25 µl of sample was added to 225 µl of wash buffer and mixed thoroughly, then 225 µl of assay diluent was added to each biochip well. 25 µl of diluted serum samples, calibration and quality controls were added to the biochip wells and taped gently. The samples were incubated in the thermo-shaker for one hour at 37 °C and 15 g, then 50 µl of conjugate was added into each biochip well and tapped gently. After this, the samples were incubated in the thermo-shaker for one hour at 37 °C and 15 g. 250 µl of signal reagent EV805 (Luminol and Peroxide, 1:1) was added to each biochip well, and wash buffer was then used to wash the biochip well four times. The Biochip well was filled with wash buffer and then uploaded into the Evidence analyser to take an image of each biochip well. Subsequently, adhesion molecules were quantified automatically using generated calibration curves (Evidence Investigator Software) (Molloy et al., 2005; FitzGerald et al., 2008; Fabre et al., 2005).

2.2.12 Calculations and statistical analysis
The details of the statistical analyses are specific to each study and are described in each specific chapter. Statistical analysis of the results was performed using either GraphPad Prism version 6 for Windows (San Diego, CA, USA) or SPSS statistical analyses software (SPSS, version 22 for windows, Chicago, Illinois). All outcomes of the human studies were assessed for normality using the Kolmogorov-Smirnov test (K-S test) and expressed as a mean ± one standard error of the mean (SEM). In the GI study the incremental area under the glucose curve (iAUC) for the reference glucose
drink was calculated as recommended by WHO (1998). Repeated measures two-way ANOVA were used to analyse the difference in the mean of fasting glucose, insulin, lipid profiles and inflammatory markers in all visits studies. All data were examined using a two-tailed approach with a level of $P < 0.05$ considered as significant.
CHAPTER THREE
Chapter Three

Radical scavenging and antioxidant activity of cinnamon supplement samples

3.1 Introduction

Reactive oxygen species (ROS) are highly damaging reactive molecules that include free radicals. A free radical contains an unpaired electron in its structure and an example of this is hydroxyl radical (OH•) (Poljšak et al., 2013). ROS, such as the superoxide radical (O2•−), H2O2 and the hydroxyl radical (OH•), are produced during normal metabolism and play a physiological role in cell signalling, however, the concentration of ROS can be increased under environmental stress and radiation, which can cause damage to the cell, and in specific circumstances, can lead to disease (Valko et al., 2007). When there is an imbalance between ROS production and the antioxidant cell defences, this can lead to an excessive amount of free radicals, which can cause cellular damage associated with many diseases (Sánchez-Gallego et al., 2011). Antioxidant compounds are substances that can break the free radical chain reaction and inhibit the production of free radicals and act as oxygen scavengers (Gülçin et al., 2005; Frankel and German, 2006). In humans, antioxidants are either produced in the body (endogenous) or are derived from the diet, which may have a direct effect on antioxidant status (Pham-Huy et al., 2008).

The total antioxidant capacity of food measures the ability of food components to scavenge free radicals (Pellegrini et al., 2003). The antioxidant activity can be measured by a variety of methods, such as DPPH radical scavenging and reducing power (Jimenez and Calixto, 2008; Huang et al., 2005). The most popular synthetic antioxidants used in the food industry are butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), but there are concerns with these synthetic antioxidants as they hepatic toxic effects (Lanigan and Yamarik., 2001). BHA and BHT are often used as standards in order to compare the antioxidant capacity of food derived substances (Nićiforović et al., 2010; Poljšak and Raspor, 2008).

Herbs and medicinal plants have been identified as sources of phytochemicals, such as polyphenolic and phenolic compounds. Some phytochemicals have powerful antioxidant activities, which may play a role in antioxidant defence in plants (Liu, 2003;
Dragland et al., 2003; Lichtenstein et al., 2006). Polyphenols are widely distributed in
plants, and are found in fruits, seeds, and herb barks; and many phenolic compounds
have been reported to have antioxidant activity (Olchowik et al., 2012). The ability of
phenols and polyphenols to act as antioxidants comes from their structure, they have
one or more aromatic rings with one or more hydroxyl groups that can donate a
hydrogen atom, which then reacts with a free radical and consequently the oxidation
reaction is stopped (Bondet et al., 1997; Dai and Mumper, 2010).

Cinnamon contains many polyphenolic compounds, including flavonoids,
anthocyanins and tannins, which increase the antioxidant activity of the products
derived from cinnamon that may offer beneficial health properties (Helal et al., 2014).
Dragland et al. (2003) have determined the total antioxidant activity in several
medicinal herbs, and C. cassia was shown to have a high total antioxidant activity
(120.2 mmol/100 g). They determined the antioxidant activity using the ferric reducing
antioxidant power of plasma (FRAP) assay, which is based on measuring the
absorption changes that appear with the reduction of ferric (Fe$^{3+}$) ions to the ferrous
ion (Fe$^{2+}$) in the presence of antioxidants (Dragland et al., 2003). The results showed
cinnamon herbal medicines have strong antioxidant properties, which may be derived
from the polyphenolic compounds they contain (Hamidpour et al., 2015). Studies have
shown there is an inverse relationship between an antioxidant rich diet and human
disease (Yildirim et al., 2001), however, there is conflicting evidence concerning the
potential benefit of higher intakes of single antioxidants, for instance, tocopherol
supplementation did not decrease the risk of CVD (Berger et al., 2012).

3.2 Study aims

The aims of this study were:

1. To prepare water and acetone/water extracts of four commercially available
cinnamon supplements.
2. To determine the total polyphenol and tannin content of the four cinnamon
supplement extracts.
3. To determine the total condensed tannin (proanthocyanidin) contents in
Cinnamomum cassia (C. cassia) and Cinnamomum zeylanicum (C. zeylanicum)
extracts.
- To determine the phenolic composition of *C. cassia* and *C. zeylanicum* using HPLC and LC-MS.
- To determine the antioxidant properties of water and 75 % acetone *C. cassia* and *C. zeylanicum* supplement extracts by using three different methods (as follows):
  - The radical scavenging method determined by DPPH and H$_2$O$_2$ scavenging of the cinnamon types at different concentrations.
  - Metal chelating activity of the different cinnamon types at a range of concentrations.
  - Determining the reducing power for each cinnamon extract at range of concentrations.

### 3.3 Methods

#### 3.3.1 Preparation of cinnamon supplement extracts

This study focused on cinnamon supplements which could be readily purchased from health shops and consumed in capsule form. Four cinnamon supplements were used in this study. The cinnamon samples were:

- Cinnamon bark (Bio-Health® *Cinnamomum zeylanicum*), 350 mg, Bio-Health Limited, Rochester, Kent, UK. (*C. zeylanicum*).

- Cinnamon bark (Health Aid® *Cinnamomum zeylanicum*) 850 mg, Health Aid Limited, Middlesex, UK. (*C. zeylanicum* H.).

- Cinnamon bark (Puritan's Pride's® *Cinnamomum cassia*) 500 mg, Holbrook, USA. (*C. cassia*).

- Cinnamon bark (Solgar® *Cinnamomum cassia*) 500 mg, Solgar Vitamin and Herb, USA. (*C. cassia* S.).

Each cinnamon sample was extracted with deionised water and 75 % acetone as described in Chapter 2 (section 2.2.2).

#### 3.3.2 Determination of total phenolic content

The cinnamon extracts were freshly prepared (section 2.2.2, Chapter 2) and triplicate analyses were performed to determine total phenolic content (section 2.2.3, Chapter 2).
3.3.2.1 Standards

A 7 mg/ml gallic acid (GA) standard stock solution was prepared and then diluted with deionised water to the following concentrations: 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 mg/ml (w/v). The standard curve was used to calculate the total phenolic content in cinnamon samples using the following formula: $Y = 0.8859X$, $R^2 = 0.995$.

The standard concentrations were prepared in triplicate.

3.3.3 Determination of hydrolysable tannin content

The analysis was performed as described previously (section 2.2.4).

3.3.3.1 Standards

A 0.1 mg/ml tannic acid stock standard solution was prepared and diluted with deionised water to the following concentrations: 0.025, 0.05, 0.1, 0.2, 0.3, 0.4 mg/ml (w/v). A standard curve was used to calculate the hydrolysable tannin content in cinnamon samples using the following formula: $Y = 0.7381X$, $R^2 = 0.996$.

3.4 Results

3.4.1 Determination of total phenols

Standard curves of gallic acid were produced (Figure 3.1) in order to estimate the total polyphenolic content in cinnamon extracts. The curve produced had a high $R^2$ value showing the points are a good fit.

![Figure 3.1: Gallic acid standard curve. Standard curve represented as a mean ± SD of gallic acid (0-0.600 mg/ml) for a total polyphenolic content determination. $R^2 = 0.995$.](image-url)
3.4.1.1 Total phenolic content of cinnamon samples extracted with 75 % acetone

The total phenolic contents of *C. cassia* and *C. zeylanicum* showed some statistically significant differences between the samples (Figure 3.2). The total phenolic contents of *C. cassia* and *C. zeylanicum* H. were similar as they were estimated to be 105.6 mg and 101.81 mg, respectively, estimated in terms of gallic acid equivalent/g of dry cinnamon. Furthermore, *C. cassia* S. had a slightly higher content (48.89 mg estimated of GAE/g) than *C. zeylanicum* (39.63 mg estimated of GAE/g), both of which were lower than *C. cassia* (Figure 3.2). These results were confirmed by the analyses at the Plant for Human Health Institution in North Carolina State University, United States, which also determined the total phenolic contents of *C. cassia* and *C. zeylanicum* to be 89.74 ±11.68 and 28.44± 2.91 mg estimated of GAE/g, respectively. Overall these results show how the different commercial supplements vary in their phenolic content, which may be related to how effective they are as treatments.

![Figure 3.2: Total phenols in cinnamon samples extracted by 75% acetone. Values are mean ± SD (n = 3). Comparisons of means were made using a one-way ANOVA followed by Bonferroni test (** =P<0.0001, * =P<0.05).](image-url)
3.4.1.2 Total phenolic content in cinnamon samples extracted using deionised water

The results for the extraction with deionised water (Figure 3.3) showed that *C. cassia* had the highest total polyphenolic content (30.65 mg GAE/g) among the cinnamon samples analysed, which is the same as in the acetone extraction. The total phenolic contents of *C. cassia*, *C. zeylanicum*, *C. cassia* S and *C. zeylanicum* H were: 30.65, 25.33, 27.89 and 19.63 mg of GAE/g, respectively (Figure 3.3), which are all lower than the acetone extraction. Overall these results suggest the 75 % acetone was a more effective solvent for extracting total phenols compared with water. The total phenolic contents in cinnamon samples extracted by water were almost three times lower compared with using 75 % acetone.

![Figure 3.3](image)

**Figure 3.3:** Total phenols in cinnamon samples extracted by deionised water. Values are mean ± SD (n = 3). Comparisons of means were made using a one-way ANOVA followed by Bonferroni test (*** = P<0.0001, * = P<0.05).
3.4.2 Determination of hydrolysable tannin content

Standard curves for tannic acid were produced (Figure 3.4) and the calculated values were subtracted from the total polyphenolic content. Tannin contents are expressed as mg/g of tannic acid equivalents (TAE).

![Tannic acid standard curve](attachment:Figure_3.4.png)

Figure 3.4: Tannic acid standard curve. Standard curve represented as a mean ± SD of tannic acid (0 - 0.400 mg/ml) for a total tannin content determination. $R^2 = 0.996$.

3.4.2.1 Total (hydrolysable) tannin determined in cinnamon samples extracted using 75 % acetone (v/v)

The hydrolysable tannin content (Figure 3.5) of *C. cassia* was two times higher (65.69 mg of TAE/g) than that present in *C. zeylanicum* (26.79 mg of TAE/g) ($p < 0.0001$).
3.4.2.2 Total tannin cinnamon samples extracted by deionised water

The water based extraction (Figure 3.6) showed that *C. cassia* had a higher level of TAE/g (23.01 mg of TAE/g) than that of *C. zeylanicum* (18.90 mg of TAE/g), which is a similar pattern to that observed for the acetone extraction. As with the phenolic content, the tannin content was higher in cinnamon samples extracted with 75% acetone compared to those extracted with water, again showing that acetone is more efficient at extraction.
Figure 3.6: Tannin content in cinnamon samples extracted by deionised water. Results are mean ± SD (n = 3). Comparisons of means were investigated using unpaired t-test (* = P<0.05).

3.4.3 Determination of condensed tannin (proanthocyanidin) contents

The DMAC method was carried out in a 96-well plate to determine the proanthocyanidin content (section 2.2.6.3). Furthermore, collaborators from NCSU (Plant for Human Health Institution), United States, determined the proanthocyanidin content and composition in *C. cassia* and *C. zeylanicum*. The results (Figure 3.7) showed there was 41.53 ± 2.70 mg of procyanidin A2/g equivalents in *C. cassia* and 11.42 ± 1.60 mg of procyanidin A2/g equivalents in *C. zeylanicum*.

The results showed that the cinnamon extracts were rich in polyphenols, and condensed and hydrolysable tannins. Therefore, several methods for measuring antioxidant activity were used to determine the antioxidant activity of *C. zeylanicum* and *C. cassia* preparations.
Figure 3.7: Proanthocyanidin content in *C. cassia* and *C. zeylanicum* samples. Results are mean ± SD (n = 3). Comparisons of means were determined using unpaired t-test (***=P<0.0001).

### 3.4.4 HPLC and LC-MS analysis

The cinnamon samples were analysed using HPLC and LC-MS (section 2.2.8 and 2.2.9) after 75 % acetone was used to extract *C. zeylanicum* and *C. cassia*. The results suggest that both cinnamon type extracts showed a similar profile.

Normal phase HPLC with fluorescence detection was able to separate the proanthocyanidin components in cinnamon extracts according to their degree of polymerisation. The data from the HPLC (Figure 3.8) revealed that both cinnamon types (*C. cassia* and *C. zeylanicum*) contain flavanol monomers (catechin and epicatechin), procyanidin dimers (types A and B), trimers (A-type), tetramers, pentamers, hexamers, heptamers, octamers and polymers. The peak intensities for the procyanidin A-type trimers reveal a high content in both *C. cassia* and *C. zeylanicum*, with a higher intensity in the *C. cassia* trace. Hence, *C. cassia* and *C. zeylanicum* may primarily contain A-type procyanidin as these are the main peaks in the HPLC trace.
Figure 3.8: Normal Phase HPLC showing the retention times for *C. cassia* and *C. zeylanicum* and the procyanidin standards.

The concentration of individual proanthocyanidins was calculated according to their degree of polymerisation (DP) by NP-HPLC. The results (Figure 3.9) show high concentrations of procyanidin polymers with degrees of polymerisation of 3 and 4 units, and polymers with degrees of polymerisation more than 10. It is shown also that concentrations of proanthocyanidins are higher in *C. cassia* in comparison to *C. zeylanicum.*
Catachin (CA), epicatachin (EC), procyanidin dimers type A2 (dimer A2), procyanidin dimers type B2 (dimer B2). Procyanidin degree of polymerisation (DP) varies from DP3 to DP10.

**Figure 3.9:** Concentrations of individual proanthocyanidins by NP-HPLC as procyanidin A2 dimer equivalents. Proanthocyanidin concentrations shown according to their degree of polymerization.

The concentrations of procyanidin type A and B dimers, trimers and tetramers, were evaluated by extracted ion chromatogram (EIC) analysis (Figure 3.10). Procyanidin A and B types were found to be the main content in *C. cassia* and *C. zeylanicum*, with dimers also being detected strongly in both. This figure reveals the presence of two types of procyanidin type B dimers in *C. cassia*, while *C. zeylanicum* had only one type. Also, two types of procyanidin type A trimers were found in *C. cassia*, whereas in *C. zeylanicum* only one type A trimer was found, and the presence of two major A type tetramers was found at different concentrations in both cinnamon types.
Figure 3.10: Extracted ion chromatograms (EIC) of proanthocyanidin dimers, trimers and tetramers in *C. cassia* and *C. zeylanicum*.

The *C. cassia* and *C. zeylanicum* extracts were analysed in the positive and negative ion modes using ESI-IT-TOF-MS (Figure 3.11). In the positive ion mode, for both types of cinnamon the major procyanidins [M+1]+ were: dimer A-type (m/z 577), dimer B type (m/z 579), trimer A-type (m/z 865), tetramer A-type (m/z 1153), and pentamer A-type...
(m/z 1441). Whereas, in the negative ion mode the major procyanidins [M-1]- were: dimer A-type (m/z 575), dimer B type (m/z 577), trimer A-type (m/z 863), tetramer A-type (m/z 1151), pentamer A-type (m/z 1439) for both types of cinnamon.

**+ve ion mode**

**-ve ion mode**

![ESI-IT-TOF-MSS can spectrum of C. cassia and C. zeylanicum extracts in the positive and negative ion modes.](image)

**Figure 3.11:** ESI-IT-TOF-MSS can spectrum of *C. cassia* and *C. zeylanicum* extracts in the positive and negative ion modes.

### 3.4.5 DPPH radical scavenging activity

The DPPH assay serves as an indirect measurement of the ability of the extracts or pure samples to inhibit free radicals. The purple DPPH radical exists as a stable free radical and has an absorbance wavelength of 515 nm, and when an antioxidant compound donates H⁺ to the DPPH molecule, it is reduced, forming a colourless compound. The calculation of the percentage of DPPH radical scavenging (2.2.10.1) was converted into the percentage of inhibition caused by both cinnamon types, extracted in 75 % acetone and deionised water (Figure 3.12).
3.4.5.1 DPPH radical scavenging activity of *C. cassia* and *C. zeylanicum* extracted by 75 % acetone and deionised water

The DPPH radical scavenging effect increased with increasing concentrations of both cinnamon varieties and solvent (Figure 3.12). The radical scavenging activity of *C. cassia* extracted by 75 % acetone was significantly higher than that of *C. zeylanicum*. The percentage of inhibition caused by *C. cassia* extracted with 75 % acetone was 36.07 %, 62.90 % and 71.69 %, respectively, for concentrations of 0.050, 0.100 and 0.150 mg/ml. However, the scavenging activity found in *C. zeylanicum* at the same concentrations was 10.51 %, 13.99 % and 19.53 % (Figure 3.12). The radical scavenging activities of the *C. cassia* and *C. zeylanicum* extracted with water were quite similar to each other (Figure 3.13), but the values were lower than the acetone extracted cinnamon. Furthermore, no significant difference was found between *C. cassia* and *C. zeylanicum* which had been extracted with water. Overall these results follow a similar trend that was seen for the other extraction comparisons, namely that the acetone is more efficient at extracting polyphenols, and *C. cassia* has a higher activity due to its higher polyphenol content.

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**Figure 3.12**: DPPH radical scavenging activity of cinnamon extracted by 75 % acetone. Results are mean ± SD (n = 3). Comparisons of means were detected using unpaired t-test (* =P < 0.05).
Figure 3.13: DPPH radical scavenging activity of cinnamon extracted by deionised water. Results are mean ±SD (n = 3). Comparisons of means were detected using unpaired t-test (* =P<0.05).

The radical scavenging activity of cinnamon extracts at 0.1 mg/ml was individually compared to that of 0.1 mg/ml of BHT and trolox (Figure 3.14). The DPPH radical scavenging capacity of BHT was not significantly different to that of *C. cassia* extracted with 75 % acetone, however, it was significantly different compared with the other cinnamon extracts (Figure 3.14). Interestingly, the DPPH radical scavenging activity of the *C. cassia* acetone extract (Figure 3.15) was significantly higher (62.90 %) compared with trolox (34.99 %), whereas the radical scavenging activity of trolox was significantly higher compared with the 75 % acetone *C. zeylanicum extract*, and the water *C. cassia* and *C. zeylanicum* extracts. Overall these results suggest that *C. cassia* extracted by acetone has a higher antioxidant activity than trolox and all the other preparations, which correlates with the high polyphenol concentrations observed in the previous experiments.
Figure 3.14: Comparison between DPPH radical scavenging activity of acetone and water *C. cassia* and *C. zeylanicum* extracts with BHT at 0.1mg/ml. Values are mean ± SD (n = 3). Comparisons of means were made using a one-way ANOVA followed by Bonferroni test (** *=P < 0.0001).

Figure 3.15: Comparison between DPPH radical scavenging activity of acetone and water *C. cassia* and *C. zeylanicum* extracts with trolox at 0.1mg/ml. Values are mean ± SD (n = 3). Comparisons of means were made using a one-way ANOVA followed by Bonferroni test (** *=P < 0.0001).
3.4.6 Scavenging of hydrogen peroxide

The capacity of cinnamon extracts to scavenge H$_2$O$_2$ was measured at 230 nm (2.2.10.2), as plant polyphenols have the ability to donate an electron to H$_2$O$_2$ and convert it to H$_2$O (Nabavi et al., 2008).

The results showed that the *C. cassia* and *C. zeylanicum* 75 % acetone extracts (Figure 3.16) strongly inhibited the activity of H$_2$O$_2$ compared with the water based extracts (Figure 3.17), and the *C. cassia* 75 % acetone extracts were significantly ($P < 0.05$ for all) more active than the *C. zeylanicum* 75 % acetone extracts. The percentage of inhibition of H$_2$O$_2$ was 95.34 %, 96.06 %, 97.33 % for *C. cassia* acetone extracts (0.050, 0.100, 0.150 mg/ml, respectively). However, the ability of the *C. zeylanicum* acetone extract to scavenge H$_2$O$_2$ at concentrations of 0.050, 0.100, 0.150 mg/ml were 89.34 %, 92.55 %, 94.12 %, respectively (Figure 3.16). There were no significant differences between the water based *C. cassia* and *C. zeylanicum* extracts (Figure 3.17). The difference between the water and acetone extractions is as expected due to the lower concentration of polyphenols present in the water extractions.

![Figure 3.16: H$_2$O$_2$ scavenging activity of cinnamon extracted by 75% acetone. Concentrations were 0.05, 0.100 and 0.150 mg/ml. Results are mean ± SD (n = 3). Comparisons of means were investigated using unpaired t-test (* =P<0.05).](image-url)
BHT and trolox at 0.1 mg/ml were used as controls to compare with 0.1 mg/ml cinnamon extracts. The percentages for the scavenging activities of *C. cassia* and *C. zeylanicum* acetone extracts were not significantly different when compared with BHT (Figure 3.18). However, the *C. cassia* (96.06 %) and *C. zeylanicum* (92.55 %) acetone extracts scavenged H$_2$O$_2$ radicals to a significantly ($P < 0.0001$) higher level than trolox (48.73 %) (Figure 3.19). Water extracts of both cinnamon types showed significantly lower inhibitory activities of H$_2$O$_2$ compared with both BHT and trolox at 0.1 mg/ml. Overall this suggests that these concentrations of the acetone cinnamon extracts are better scavengers than the controls.
Figure 3.18: Comparison between $\text{H}_2\text{O}_2$ scavenging activity of acetone and water cinnamon sample extracts with BHT at 0.1mg/ml. Values are mean ± SD ($n = 3$). Comparisons of means were made using a one-way ANOVA followed by Bonferroni test (*** =P<0.0001).

Figure 3.19: Comparison between $\text{H}_2\text{O}_2$ scavenging activity of acetone and water cinnamon sample extracts with trolox at 0.1mg/ml. Values are mean ± SD ($n = 3$). Comparisons of means were made using a one-way ANOVA followed by Bonferroni’s test (*** =P<0.0001).
3.4.7 Metal chelating activity

The method was described in section (2.2.10.3). During ferrous ion chelation, the Fe$^{2+}$ ferrozine complex formation can be inhibited by the presence of antioxidant compounds. A chelating agent that has the ability to inhibit the formation of Fe$^{2+}$ ferrozine complex is considered to be Fe$^{2+}$ chelating (Ebrahimzadeh et al., 2010). The results show the percentages of ferrous chelating activity for each type of cinnamon extract (either by 75% acetone (Figure 3.20) or by water (Figure 3.21) were relatively similar for the concentrations investigated. In addition, there was no significant difference between the acetone and water based extracts of *C. cassia* and *C. zeylanicum*. This was due to the low level of chelating that occurs in both types of cinnamon.

![Graph showing metal chelating activity](image)

**Figure 3.20:** Metal chelating activity of cinnamon extracts by 75% acetone. Concentration were 0.05, 0.100 and 0.150 mg/ml. Results are mean ± SD (n = 3). Comparisons of means were investigated using unpaired t-test (* = P<0.05).
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The ferrous chelating activity of EDTA (Figure 3.22), which was used as control, was significantly higher than that of the cinnamon extracts. The ferrous chelating activity of EDTA, acetone extracted \( C. \text{ cassia} \), acetone extracted \( C. \text{ zeylanicum} \), water extracted \( C. \text{ cassia} \), and water extracted \( C. \text{ zeylanicum} \) extracts were 98.39 %, 8.67 %, 6.61 %, 4.55 % and 2.80 %, respectively. Overall this shows that the cinnamon extracts had a poor chelating activity compared to the control, which suggests chelation is not the main method that produces the beneficial effects of cinnamon.

**Figure 3.21:** Metal chelating activity of cinnamon extracts by deionised water. Concentration were 0.05, 0.100 and 0.150 mg/ml. Results are mean ±SD (n = 3). Comparisons of means were investigated using unpaired t-test (* =P<0.05).
3.4.8 Reducing power assay

Method was described in section (2.210.4). The reducing power of a compound is based on its capacity to donate an electron, which is a mechanism of phenolic antioxidant action (Hinneburg et al., 2006). The presence of an antioxidant compound in an extract leads to the reduction of Fe$^{3+}$ (ferricyanide complex) to Fe$^{2+}$ (ferrous) through the donation of an electron (Nabavi et al., 2008). The results for the investigation of the reducing power of the cinnamon extracts (Figure 3.23 and Figure 3.24) showed they increased with increased concentration for all extracts, but the reducing power of the C. cassia acetone extract was significantly ($P < 0.001$) higher compared with that of C. zeylanicum (Figure 3.23). In addition, the C. cassia water extract had higher reducing activity than that of C. zeylanicum, but this was not significant (Figure 3.24). These results show that the cinnamon extracts have a reducing power, with the acetone extracts having a higher activity than the water extracts, which is as expected based on the previous results in this study. The results also agree with the other investigations of cinnamon extract activity, as the C. cassia has a higher activity than the C. zeylanicum, which fits with the differences observed in polyphenol content between the extracts.
Figure 3.23: Reducing power of different concentrations from cinnamon samples extracted by 75% acetone. Each value is the mean ± SD of three measurements. Comparisons of mean were made using unpaired t-test (** = P<0.001).

Figure 3.24: Reducing power of different concentrations from cinnamon samples extracted by water. Each value is the mean ± SD of three measurements. Comparisons of mean were made using unpaired t-test.
3.5 Discussion

Many previous studies have reported the potential health benefits of procyanidins, for example feeding male rats (Zucker Fa/fa) procyanidins extracted from grape seed (345 mg/kg feed) for 19 weeks, significantly reduced the high levels of CRP, which are linked with inflammation (Terra et al., 2009). Andújar et al. (2012) reviewed the potential benefits of cocoa polyphenols, and they reported that 58 % of cocoa is consists of procyanidin. Grassi et al. (2008) reported from a randomised crossover trial, that feeding 19 hypertensive patients flavanol-rich dark chocolate (100 g/day for 15 days) significantly decreased their systolic and diastolic blood pressure. Our data identified the procyanidins type A and B in cinnamon and other studies have confirmed their health benefits. Therefore, this study investigated the antioxidant activity of cinnamon samples.

According to our knowledge, this is the first study compare the antioxidant activities of cinnamon extracted with acetone and water, and to compare the antioxidant activities of two types of cinnamon supplements (C. cassia and C. zeylanicum). Furthermore, the first study determined hydrolysable and condensed tannin in cinnamon. The total phenol levels in our study were relatively high compared to the results of the North Carolina State University (NCSU), United States, who also analysed the polyphenol composition of different cinnamon samples using HPLC and LC-MS. The total phenol levels of C. cassia and C. zeylanicum extracted by 75 % acetone in our lab were 105.6 mg and 39.63 mg, and in NCSU were 89.74 mg and 28.44 mg respectively, of gallic acid equivalent/g of dry cinnamon. Both laboratories used the same extraction solvent, however, NCSU used a different extraction method. The acetone cinnamon extracts were high in total phenols and tannins when compared with the water cinnamon extracts. This is due to the solubility of the plant and herb polyphenols being influenced by the solvent used for the extraction process; in general, acetone and water mixes are more efficient than acetone alone for the extraction of phenolic substances from herbal medicines (Meneses et al., 2013). In addition, solvent polarity plays a key role in increasing phenolic solubility, which relates to the high total phenolic content and antioxidant activity seen in the acetone cinnamon extracts (Naczk and Shahidi, 2006; Barchan et al., 2014). As water has a higher polarity, it is not as efficient at extracting phenols because these compounds are often more soluble in organic solvents which are less polar than water (Meneses et al., 2013). This observation has been confirmed
by Su et al., (2007), who showed that a 50 % acetone solvent produced better extractions than another more polar alcoholic solvent (85 % methanol). Nevertheless, Tatiya et al. (2011) report that 70 % acetone was efficient for the extraction of tannin. Furthermore, for the extraction of procyanidins, acetone has been shown to possess the highest extraction efficiency, followed by ethanol and methanol (Lazarus et al., 2001). Therefore, for this study 75 % acetone was used to extract the phenolic compounds, which was more efficient than water. In addition, deionised water was used to extract the cinnamon samples in order to have a sample that was similar to that experienced by a consumer of cinnamon.

Su et al. (2007) showed that a 50 % acetone cinnamon extract has 186 mg of gallic acid equivalents/g, which was higher than our result (105.6 mg), although both studies used acetone. This might be due to their different extraction method, which used 5 g ground cinnamon extracted with 50 ml of 50 % acetone for 15 hours, which meant each one ml of solvent was used to extract 100 mg of cinnamon. Furthermore, they extracted for double the amount of time and therefore, the total phenols in Su et al. (2007) was higher compared with our study. In this study, the total polyphenols in C. cassia and C. zeylanicum water extracts were 30.65 mg and 25.33 mg of gallic acid equivalents/g of dry cinnamon, respectively, which was lower than the findings of Helal et al. (2014), who determined the total polyphenols in water C. cassia extract to be 43.8 mg of catechin equivalents/g of dry cinnamon. This difference was possibly because they used a different method to extract C. cassia. In their methods, 4 g of C. cassia was added to 200 ml of boiling water, and was then heated for 5 min below boiling point. In addition to this, they used a different standard, which was catechin, which would produce a lower result than gallic acid in the assay due to its higher molecular mass. These results are also similar to the study by Ranilla et al. (2010) who found that C. zeylanicum water extract has 35 mg of gallic acid equivalents/g of dry cinnamon. Again, this difference in total phenol content may be due to differences in the extraction methods, they added 5 g of cinnamon to 100 ml of distilled water and refluxed the mixture at 95 °C for 30 min before cooling. The extract was then centrifuged at 9300 g for 30 min, and the final supernatant was re-centrifuged at 1008 g. These studies show that the extraction method plays an important role in determining the total phenolic content measured in the cinnamon samples.
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The total phenol content in the *C. cassia* acetone extract (105.6 mg GAE/g) found in this study was similar to that of Lv et al. (2012) (101.4 mg GAE/g), and this may be due to both studies having used acetone at the same percentage, 75 % acetone. In the study carried out by Lv et al. (2012), the cinnamon was extracted using an ASE 200 system stainless steel cell (Dionex, Sunnyvale, CA), which was used for the pressurised liquid extraction of the cinnamon, which was then extracted with 75 % acetone. Their cinnamon extract was obtained via pressure and high temperature, which might have an effect on cinnamon polyphenols. While our method that was used to extract cinnamon samples was based on stirring at low temperature, which possibly has a lower impurities effect on the cinnamon polyphenols. Sardsaengjun et al. (2010) highlighted that increased temperature might increase impurities in the extract that may affect total polyphenolic content. Also, higher temperatures may cause the extraction of compounds other than polyphenols diversity (Vergara-Salinas et al., 2012).

In the acetone cinnamon extracts, the total hydrolysable tannin contents of *C. cassia* was much higher than *C. zeylanicum*, 65.69 mg and 26.79 mg (respectively) of tannic acid equivalents (TAE/g), and the water based extract of *C. cassia* also had a higher level of hydrolysable tannins (23.01 mg of TAE/g) than that of *C. zeylanicum* (18.90 mg of TAE/g), but the difference was not as large. No previous studies have been documented which determined the total hydrolysable and condensed tannin contents in cinnamon, which suggests this study is the first study measure the total hydrolysable tannin and proanthocyanidin contents of cinnamon, and again shows that acetone was a more suitable solvent for the extraction process.

It is clear from the outcome of this study that *C. zeylanicum* extracts were lower in total phenol and tannin content compared with *C. cassia*. This might be due to the polyphenol compositions obtained from the HPLC and LC-MS analyses (section 3.4.2.4). Our HPLC results show that *C. cassia* had higher levels of monomers (catechin and epicatachin), which were used as a standard, than *C. zeylanicum*. This was in agreement with Lv et al. (2012) whose HPLC data confirmed that catechin was the major phenolic compound in *C. cassia*. Their study analysed one type of cinnamon, whereas our study investigated two types, which provides more details relating to cinnamon content. Moreover, our extracted ion chromatogram (EIC) analysis data showed that *C. cassia* has two types of procyanidin type-B dimers, while *C. zeylanicum* has only one type. Also, two types of procyanidin type-A trimers were found in *C.
cassia, whereas, C. zeylanicum was found to have one type-A trimer. This result agrees with the work by Chen et al. (2012) as their HPLC chromatographic profiles analysed C. cassia and confirmed that it contained mainly procyanidins of the type-B (Chen et al., 2012; Chen et al., 2014). Furthermore, our results were consistent with those of Anderson et al. (2004), who found that their HPLC-APCI-MS ion profiling of both types of cinnamon extract showed the presence of monomers and trimers of type-A procyanidins, along with catechin, epicatechin, and procyanidins B1 and B2. Also, Anderson et al. (2004) reported that the major procyanidin oligomers in the ethanol and aqueous extracts of cinnamon were doubly linked type-A, which is in contrast to the findings of Chen et al. (2012) who reported that C. cassia contained mainly type-B procyanidins, which may be due to the extraction process they used. Our study concurs with Chen et al. (2012) and Anderson (2004). In both varieties cinnamon procyanidins type A and B exist and other studies have confirmed their health benefits; therefore, it would be useful to test the effects of cinnamon, in which LC-MS and HPLC have confirmed the presence of procyanidins, on cytokine levels and blood pressure in vivo.

In this study, the antioxidant activity of cinnamon extracts increased with concentration, which is as expected. The acetone extract of C. cassia had the highest antioxidant activity, with DPPH, H$_2$O$_2$ radical scavenging, and reducing power, compared with the acetone extract of C. zeylanicum, and both cinnamon water extracts. The acetone C. cassia extracts showed a high free radical scavenging capacity compared with BHT and trolox at 0.100 ml/ml, suggesting this extract is a strong antioxidant. In a study by Mathew and Abraham (2006), their C. zeylanicum extract showed good scavenging of DPPH radicals compared with the BHA positive control, at different concentrations (50-3125 µg/ml). At a concentration of 125 µg/ml, which was relatively similar to one of the concentrations used in this study (100 µg/ml), the scavenging capacity of their C. zeylanicum extract was 13 %, and for BHA at the same concentration (125 µg/ml), it was 25 %. However, our study found that the scavenging capacity of the acetone C. zeylanicum extract was 13.99 %, and for BHT it was 60 %, which shows the similarity in the cinnamon results. Although in the study by Mathew and Abraham (2006) the cinnamon was extracted with a different solvent (methanol) under stirring, their results support and agree with our result in showing that acetone C. zeylanicum extract was effective at scavenging DPPH radicals. Furthermore, Dudonné et al. (2009) highlighted that a water C. zeylanicum extract inhibited 84.43 % of DPPH radicals. In their study
they extracted 125 g of cinnamon with distilled water at 50 °C under agitation and after filtration, the water was removed in a Buchi R124 rotary evaporator. However, their study did not mention the cinnamon extract concentration used to scavenge DPPH radicals, and it was not compared with a positive control making it harder to compare the results to our study. In another study, a *C. zeylanicu* water extract at a concentration of 50 mg/ml inhibited 45 % of DPPH radicals (Ranilla et al., 2010). Comparing our result with their outcome, our *C. zeylanicum* water extract at 0.100 mg/ml inhibited 9.07 % of DPPH radicals, and although the concentrations compared are not similar, it is evident that *C. zeylanicum* water extract at a low concentration (0.100 mg/ml) is active in scavenging DPPH radicals. Nevertheless, Yang et al. (2012) state that *C. cassia* extracted with 95 % ethanol scavenged 85 % of DPPH radicals when at a concentration of 0.073 mg/ml, which is similar to our result as at a low concentration (0.100 mg/ml), the acetone extract of cinnamon performed more effectively in scavenging DPPH radicals (62.90 %) than in the study by Yang et al. (2012). Also, in their study they did not compare their results with a positive control, but overall it seems that acetone is a more efficient extraction solvent than ethanol. This has also been confirmed by Lazarus et al. (2001) who emphasised that acetone has been shown to possess the highest extraction efficiency of polyphenols, in particular procyanidin, followed by ethanol and methanol.

In a study by Singh et al. (2007), they tested the activity of essential oils and oleoresins of the bark of *C. zeylanicum* in scavenging H$_2$O$_2$ radicals. The highest concentration (25 µl/5 ml) of essential oils and oleoresins of the bark of *C. zeylanicum* showed results of 79.6 % and 78.6 %, respectively, and were compared with the synthetic antioxidant BHT (83.2 %). These results were not significantly different compared with the positive control BHT, but their result corresponds with our H$_2$O$_2$ result, although they used a different extraction method and different concentrations. In addition, the chelating effects of oleoresins of *C. zeylanicum* bark at 25 µl/5 ml were 38.5 %, whereas the EDTA chelating ability was 90.4 % (Singh et al., 2007), which also agrees with our results, which showed that cinnamon extracts could chelate ferrous ions but they were not as effective as EDTA.

In this study, the reducing power of cinnamon extracts increased with concentration as expected. This means that cinnamon extracts act as electron donors and can react with free radicals to convert them to more stable products. Singh et al. (2007)
confirmed our findings that *C. zeylanicum* extract produced a high reducing power, and their results increased with the concentration of oleoresins of bark *C. zeylanicum*, which ranged from 5 -25 µl/5ml. Overall, cinnamon is a natural herb that might donate an electron or hydrogen to a DPPH• radical make it a stable molecule. The results for *C. cassia* acetone extract were significantly higher than trolox, but not significantly higher than BHT. Therefore, cinnamon could be used as an alternative to synthetic antioxidants, and these results show cinnamon has health benefits when used as an antioxidant natural herb. The antioxidant activity of the cinnamon extracts may be due to the presence of proanthocyanidins, which are potent free radical scavengers and contributors to the health benefits of herbs, in particular cinnamon (Prior and Gu., 2005).

### 3.6 Conclusions

The results of this study have shown that acetone as a solvent was more efficient than water in extracting polyphenols and tannins in both cinnamon types, and *C. cassia* had the highest total polyphenol and tannin content. This suggests that acetone as a solvent was more efficient than water in increasing the solubility of cinnamon phenolic compounds and tannins in *C. cassia* and *C. zeylanicum*. Furthermore, the antioxidant studies confirm that *C. cassia* and *C. zeylanicum* extracts show antioxidant activity. The antioxidant activity of 75 % acetone extracted *C. cassia* was found to be higher than acetone extracted *C. zeylanicum*, and these cinnamon extracts had the ability to scavenge free radicals present from DPPH and H₂O₂. The comparison of cinnamon extract antioxidant activities with the common antioxidant compounds BHT and trolox showed that *C. cassia* has a significantly higher activity than trolox and non-significantly higher than BHT. This activity might be due to the total polyphenol and tannin contents that are present in this cinnamon type detected by HPLC and LC-MS analysis, which showed that have *C. cassia* and *C. zeylanicum* have two types of type B procyanidins, *C. cassia* has two types of procyanidins, type A and B, while *C. zeylanicum* has one type of type A and B. These positive results were shown more clearly and strongly in *C. cassia* compared with *C. zeylanicum*. These results show that cinnamon contains high levels of polyphenols which could affect the digestion and absorption of nutrients, therefore the next chapter investigated the effect of cinnamon on the RAG and SAG of a starch rich test meal in *vitro*.
Determination of rapidly available glucose (RAG) and slowly available glucose (SAG) values of a starch rich food in the presence of *C. cassia* and *C. zeylanicum* supplements

### 4.1 Introduction

Carbohydrates play a major role in human diets, constituting approximately 50% of the daily food intake (Scientific Advisory Committee on Nutrition (SACN), 2015). Carbohydrates are digested by α-amylase, which is secreted by the pancreas and salivary glands, and α-glycosidase, which is released from the intestinal epithelia (Satoh et al., 2015). Carbohydrates can be classified according to their chemical structure, however, this is not a useful approach in terms of their physiological effects; therefore Englyst et al. (1999) developed a novel enzymatic method to determine carbohydrate digestion *in vitro*, and linked this with the glycaemic response. This method classifies carbohydrates *in vitro* into rapidly available glucose (RAG), which is the rapidly released glucose derived from carbohydrate digestion (within 20 min), and slowly available glucose (SAG), which is the glucose released from carbohydrate more slowly (between 20 min and 120 min). In 1981 Jenkins and colleagues introduced the glycaemic index (GI), which distinguishes carbohydrates according to their physiological impact on blood glucose levels. This term ranks foods according to how much blood glucose is increased in response to a portion of food containing 50 g of available carbohydrate. In addition, the GI has been authorised for use by the FAO/WHO (1997), and many researchers and organisations recognise the important role of GI in the prevention and treatment of diseases such as obesity, diabetes mellitus and cardiovascular disease (Ludwig and Eckel, 2002; Mann et al., 2007; Wang et al., 2014). Low GI diets have beneficial effects on plasma LDL, and can modulate plasma triglycerides and HDL in diabetes, obesity and in healthy people (Liu et al., 2000; Toeller et al., 2001; Augustin et al., 2015).

Studies have shown that the RAG values for a range of carbohydrate rich foods correlate strongly with their GI value *in vivo* (Englyst et al., 1996). Therefore, Englyst et al. (1999) suggest that RAG is linked to the rapidly digested carbohydrates, which
quickly raise blood glucose levels and insulin responses. However, SAG relates to the release of glucose from slowly digested carbohydrates, which results in a slower elevation of blood glucose levels and associated insulin responses. This suggests that the determination of the RAG value of a specific carbohydrate rich food in vitro could be a good indicator of the GI value of the same food in vivo, and this could help increase the understanding of how different food types and different food processing can impact on blood glucose and insulin levels (Englyst et al. 1999). The Englyst et al. (2000) method used to determine RAG and SAG values is based on enzymatic procedures which break down the carbohydrate.

Studies have suggested that foods which are rich in phytochemicals, such as gums and polyphenols, show a low glycaemic response and a low RAG value in vitro (Englyst and Englyst, 2005). For example, ingesting 150 g of berries, a rich source of polyphenols such as proanthocyanidins, with sucrose (35 g), significantly reduced the postprandial glycaemic response (Hanhineva et al., 2010).

Cinnamon is a plant rich in phytochemicals, particularly polyphenols, which is also used as a herbal medicine (Jarvill-Taylor et al., 2001; Lu et al., 2011). Research has shown that polyphenols derived from berries may be able to inhibit the digestive enzymes involved in lipid and carbohydrate breakdown, which may be useful in reducing obesity and controlling glucose release from dietary carbohydrate (McDougall et al., 2008, 2009). These polyphenols have also been shown to inhibit the digestive enzymes α-glucosidase and pancreatic α-amylase (McDougall et al., 2005), and this enzyme inhibition may be due to the fact that the berries have high concentrations of polyphenols such as proanthocyanidins, which interact with starch digestion by binding to one or more sites of digestive proteins. This could change the surface conformation needed for an enzyme and so reduce the enzyme activity (Toda et al., 2001; Boath et al., 2012). Toda et al. (2001) reported that tannins have the ability to inhibit intestinal α-glucosidase activity. Interestingly, the inhibition of carbohydrate digestive enzymes is the target of a drug called acarbose, which is used to control blood glucose levels in type 2 diabetes patients after meals. Although acarbose is an effective treatment for modulating glucose levels in type 2 diabetes patients, it does have side effects including the fact that large amounts of undigested starch reach the colon and cause fermentation and discomfort (Boath et al., 2012). Therefore, identifying natural herbs that contain high levels of polyphenols, such as cinnamon, could be useful in terms of
producing potential health benefits, as this might in turn reduce the dose of acarbose necessary to treat patients with type 2 diabetes.

4.2 Study aims

The aims of this study are:

- To determine the effect of different doses of *C. cassia* and *C. zeylanicum* powder on RAG values in a simulated carbohydrate digestion *in vitro*.
- To determine the effect of different doses of *C. cassia* and *C. zeylanicum* powder on SAG values in a simulated carbohydrate digestion *in vitro*.
- To extract *C. cassia* and *C. zeylanicum* with deionised water (1 g/ml).
- To determine the effect of *C. cassia* and *C. zeylanicum* water extracts on RAG and SAG values *in vitro*.
- To investigate whether cinnamon extract samples inhibit carbohydrate digestive enzyme (α-glycosidase and α-amylase) activity and compare their effect with acarbose (1 g/ml).

4.3 Methods

4.3.1 Preparation of cinnamon samples

Two cinnamon supplement samples were selected for the study. They were both cinnamon bark supplements: Puritan's Pride's ® *Cinnamomum cassia* (500 mg, Holbrook, USA) and Bio-Health ® *Cinnamomum zeylanicum* (350 mg, Bio-Health Limited, Rochester, Kent, UK). The quantities of cinnamon that were used in the method, which were incubated with the hydrolytic mixture of enzymes were 32 mg, 64 mg, 96 mg, 128 mg, and 160 mg per incubation. The related concentrations of these cinnamon additions were 1.28, 2.56, 3.84, 5.12 and 6.4 mg/ml. In addition, four reference foods were studied using the RAG and SAG methods; the carbohydrate reference sample used with the different cinnamon doses was cornflakes (Englyst Carbohydrate Services Ltd, Southampton, UK). The other carbohydrate containing foods/samples were potato starch (Sigma Chemical Company Ltd, Poole, UK), white wheat flour and biscuits (Englyst Carbohydrate Services Ltd, Southampton, UK).
4.3.2 RAG and SAG measurement

For the measurement of RAG and SAG values, portions of cornflakes (0.8 g) (Englyst reference material) were weighed to the nearest ± 1 mg into 50 ml polypropylene centrifuge tubes (Corning Inc., USA) in the presence and absence of cinnamon. These were incubated for 30 minutes with a freshly prepared pepsin–guar gum solution (as described in section 2.1.4). Further procedures and incubations with the hydrolytic mixture enzymes were under controlled conditions of temperature (37 °C), pH (pH 5.2) and shaking speed, (which are described in section 2.2.1.1). At specific time points (20 and 120 min) samples (0.5 ml) were collected from the incubation tubes. The samples were then analysed for glucose to calculate the RAG and SAG values of cornflakes in the presence and absence of cinnamon samples (section 2.2.1.1.1).

Although four reference foods were tested, cinnamon samples were only added to the cornflakes because it is a popular food that is consumed at breakfast. The doses added to the starch food (cornflakes) were 32, 64, 96, 128 and 160 mg of both cinnamon types. Additional incubation tubes containing cinnamon standards and blanks were used to adjust the RAG and SAG values as appropriate. Three further reference foods were also analysed, these were cornflakes (Englyst Carbohydrate Services Ltd, Southampton, UK), potato starch (Sigma Chemical company Ltd, Poole, UK), biscuits (Englyst Carbohydrate Services Ltd, Southampton, UK) and commercial white wheat flour, purchased from ASDA. The cornflake reference food (Englyst Carbohydrate Services Ltd, Southampton, UK) was included in every batch of samples analysed as a quality control. The reference foods purchased were of the same batch number.

The coefficient of variation (CVs) of G20 and G120 for cornflakes were 3.97 % and 4.75 %, respectively. The CVs of G20 and G120 for potato starch were 4.71 % and 3.32 %, respectively. For white wheat flour the CVs of G20 and G120 were 1.55 % and 3.01 %, respectively, and for biscuits they were 3.12 % for G20 and 3.08 % for G120.

4.3.3 Preparation of acarbose

Acarbose (>95 % purity, Sigma Chemical Company Ltd, Poole, UK) was dissolved in deionised water at a concentration of 1 g/ml. Acarbose is a drug that inhibits the two main enzymes involved in starch digestion, α-amylase and α-glucosidase, and is prescribed to control blood glucose levels in type 2 diabetes patients after starch-containing meals. Acarbose was used in this experiment as a control as it inhibits α-
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amylase and α-glucosidase, which digests starch. Three concentrations were used: 12.8, 25.6 and 51.2 µg/µl.

4.3.4 Preparation of water cinnamon extracts

Cinnamon samples extracted with deionised water at 1 g/ml were used in this experiment (section 2.2.2). The same three concentrations were used as that for acarbose: 12.8, 25.6 and 51.2 µg/µl. The cinnamon extracts were freshly prepared for each experiment.

4.3.5 Calculation and statistical analyses

Values for RAG and SAG were calculated from the measured $G_{20}$ and $G_{120}$ as the following:

$$RAG (g) = G_{20}$$
$$SAG (g) = G_{120} - G_{20}$$

Results were expressed as the mean ± standard deviation. Differences between the RAG and SAG values, in terms of different doses of the same type of cinnamon, were examined by one-way analysis of variance (ANOVA) followed by Bonferroni testing between different doses and controls, which were the cornflake sample values. Values of $p \leq 0.05$ were considered significant.

4.4 Results

4.4.1 Analysis of the four reference foods (potato starch, wheat flour, cornflakes and biscuits)

The RAG (Figure 4.1) and SAG values (Figure 4.2) for the four reference foods were measured using the colorimetric manual method. As expected, cornflakes had the highest RAG and the lowest SAG values, among the reference foods, whereas potato starch had the lowest RAG value and almost the highest SAG value. White wheat flour had the highest SAG value of the reference foods. This shows that the reference foods chosen have a range of properties that can be compared to the foods supplemented with cinnamon.
**Figure 4.1:** RAG values for the four reference foods obtained from the manual colorimetric assays. Values are mean ± SD (n = 3).

**Figure 4.2:** SAG values for the four reference foods obtained from the manual colorimetric assays. Values are mean ± SD (n = 3).
The results obtained from the reference foods for RAG and SAG values using spectrophotometry were significantly different to those in the literature. However, the RAG and SAG values of cornflakes were similar to those in the literature (Table 4.1). Reference foods were used to make sure that the experiment process worked as expected and helped to pinpoint any error in the experiment that could be corrected for the next experiment. In addition, it was used to compare with the test food. The RAG value for the reference cornflakes was very similar (81.3 vs. 79.0), and also for the SAG value (4.1 vs. 2.0). Moreover, the biscuit RAG was similar to that obtained from the literature (39.9 vs. 36.1) although the SAG value was lower (16.5 vs. 26.0). On the other hand, the RAG value of the potato starch (19.6 vs. 4.0) was significantly higher ($P = 0.001$) than that in the literature (4.0). However, the SAG value was similar to that obtained in the literature (23.6 vs. 22.0).

**Table 4.1:** The RAG and SAG values for the four reference foods from the manual colorimetric assay and literature (Mean ± SD)* Englyst (2000).

<table>
<thead>
<tr>
<th>Reference Food</th>
<th>Colorimetric assay</th>
<th>Literature</th>
<th>Unpaired t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RAG</td>
<td>SAG</td>
<td>RAG</td>
</tr>
<tr>
<td>White wheat flour</td>
<td>46.3 ± 1.8</td>
<td>27.6 ± 2.3</td>
<td>35.0 ± 1</td>
</tr>
<tr>
<td>Englyst biscuit</td>
<td>39.9 ± 0.1</td>
<td>16.6 ± 2.7</td>
<td>36.0 ± 1</td>
</tr>
<tr>
<td>Potato starch</td>
<td>19.6 ± 2.2</td>
<td>23.6 ± 2.2</td>
<td>4.0 ± 1</td>
</tr>
<tr>
<td>Englyst cornflakes</td>
<td>81.3± 3.2</td>
<td>4.1 ± 1.7</td>
<td>79 ± 1</td>
</tr>
</tbody>
</table>

4.4.2 RAG and SAG values of cornflakes (Englyst reference) in the presence of different doses of *C. cassia*

The *C. cassia* powder was added to cornflakes in different doses, and the results from these incubations were compared with the RAG and SAG values of cornflakes as a control. The results (Figure 4.3) showed the RAG value of the cornflakes was reduced when the dose of *C. cassia* was increased. At the doses of 64 mg, 96 mg, 128 mg and 160 mg of cinnamon the RAG values were reduced significantly (72.1 %, $P = 0.0192$; 69.8 %, $P = 0.003$; 68.6 %, $P = 0.0016$; 64.4 %, $P = 0.0001$), compared with the control. Whereas, the SAG values (Figure 4.4) were increased when the cinnamon dose increased. Moreover, the SAG values of cornflakes with this cinnamon added at doses
of 96 mg, 128 mg and 160 mg were increased significantly. The decrease in RAG values related to the increase in SAG values.

Figure 4.3: RAG value for control and cornflakes with different doses of *C. cassia* (*n* = 3). Comparisons of means were made using a one-way ANOVA followed by Bonferroni test (* = *P* < 0.05, ** = *P* < 0.001, *** = *P* < 0.0001).

Figure 4.4: SAG value for control and cornflakes with different doses of *C. cassia* (*n* = 3). Comparisons of means were made using a one-way ANOVA followed by Bonferroni test (* = *P* < 0.05, ** = *P* < 0.001, *** = *P* < 0.0001).
4.4.3 RAG and SAG values of cornflakes (Englyst reference) in the presence of different doses of *C. zeylanicum*

Different doses of *C. zeylanicum* powder were added to cornflakes and compared with the RAG and SAG values of cornflakes as a control. The RAG values (Figure 4.5) of the cornflakes were significantly reduced by *C. zeylanicum* at doses of 64 mg, 96 mg, 128 mg and 160 mg (71.5 %, 70.5 %, 64.7 % and 64.1 %). However, the SAG values (Figure 4.6) were only increased slightly in the presence of this cinnamon, and there were no significant differences between the cinnamon concentrations. The decrease in RAG values related to the slight increase in SAG values caused by *C. zeylanicum*, but this was not significant.

![Figure 4.5: RAG value for control and cornflakes with different doses of *C. zeylanicum* (n = 3). Comparisons of means were made using a one-way ANOVA followed by Bonferroni test (* =P<0.05, ** =P<0.001, *** =P<0.0001).](image-url)
Figure 4.6: SAG value for control and cornflakes with different dose of *C. zeylanicum* \( (n = 3) \). Comparisons of means were made using a one-way ANOVA followed by Bonferroni test (NS = not significant).

4.4.4 A Comparison of RAG values of cornflakes in the presence of *C. cassia* and *C. zeylanicum* water-based extracts and acarbose at different concentrations

Water-based extracts were prepared for *C. cassia* (W.E. *cassia*), *C. zeylanicum* (W.E. *zeylanicum*) and acarbose (1 g/ml). These extracts were added at different concentrations (12.8, 25.6, 51.2 µg/ml) to cornflakes to measure their effect on RAG and SAG values. The effects of the two varieties of cinnamon were evaluated with respect to the cornflakes’ RAG values, and these were determined for each concentration and compared with the RAG value of cornflakes (control). The results showed that the RAG value of cornflakes was significantly \( (P < 0.0001) \) reduced by acarbose compared with control cornflakes, at 12.8 µg/ml (26.5 vs. 81.3 %, Figure 4.7), 25.6 µg/ml (21.0 vs. 81.3 %, Figure 4.8), and 51.2 µg/ml (14.7 vs. 81.3 %, Figure 4.9). However, there were no significant effects on the RAG values observed for either cinnamon type at any concentration. The concentration of water cinnamon extracts were perhaps too low to have an impact on RAG and SAG values. While acarbose was pure (>95 % purity) and had a significant effect on RAG and SAG values.
Figure 4.7: RAG values of cornflakes in the presence of water *C. cassia*, *C. zeylanicum* extracts and acarbose at 12.8 µg/ml (n = 3). Comparisons of means were made using one-way ANOVA followed by Bonferroni test (**=*p<0.0001).

Figure 4.8: RAG values of cornflakes in the presence of water *C. cassia*, *C. zeylanicum* extracts and acarbose at 25.6 µg/ml (n = 3). Comparisons of means were made using one-way ANOVA followed by Bonferroni test (**=*p<0.0001).
Figure 4.9: RAG values of cornflakes in the presence of water *C. cassia*, *C. zeylanicum* extracts and acarbose at 51.2 µg/ml (n = 3). Comparisons of means were made using one-way ANOVA followed by Bonferroni test (***=p<0.0001).

### 4.4.5 Comparison of SAG values of cornflakes in the presence of *C. cassia* and *C. zeylanicum* water-based extracts and acarbose at different concentrations

The effects of the two varieties of cinnamon and acarbose were evaluated with respect to the cornflakes SAG values, for each concentration, and were compared with the SAG value of the control cornflakes. The SAG value of cornflakes in the presence of acarbose was significantly (**P < 0.0001**) increased compared with the control, at 12.8 µg/ml (16.42 vs 4.12 %, Figure 4.10) and a smaller significant (**P < 0.05**) increase was observed for 25.6 µg/ml (9.11 vs 4.12 %, Figure 4.11). The SAG values for cornflakes in the presence of both cinnamon varieties were slightly increased compared with the control cornflakes (Figure 4.10, Figure 4.11 and Figure 4.12), however, these did not reach statistical significance. None of the supplements produced a significant difference in SAG at a concentration of 51.2 µg/ml. Overall these results show that acarbose had a larger effect than cinnamon, but the changes in RAG were not mirrored by similar changes in SAG.
**Figure 4.10:** SAG values of cornflakes in the presence of water *C. cassia*, *C. zeylanicum* extracts and acarbose at 12.8 µg/ml (n = 3). Comparisons of means were made using one-way ANOVA followed by Bonferroni test (***=p<0.001).

**Figure 4.11:** SAG values of cornflakes in the presence of water *C. cassia*, *C. zeylanicum* extracts and acarbose at 25.6 µg/ml (n = 3). Comparisons of means were made using one-way ANOVA followed by Bonferroni test (***=p<0.001).
Figure 4.12: SAG values of cornflakes in the presence of water C. cassia, C. zeylanicum extracts and acarbose at 51.2 µg/ml (n = 3). Comparisons of means were made using one-way ANOVA followed by Bonferroni test.

4.5 Discussion

The objective of this study was to measure the RAG and SAG values of cornflakes in the presence of C. cassia and C. zeylanicum at different doses, and to compare this with the RAG and SAG values of cornflakes as a control.

4.5.1 Reference foods (potato starch, wheat flour, cornflakes and biscuit)

The RAG and SAG values for the reference foods were significantly different compared with the literature. The difference occurred in potato, biscuit and wheat flour compared with literature were possibly due to differences in the origin, variety and season of reference foods used. On the other hand, the RAG and SAG values of cornflakes were very similar to literature. Therefore, cornflakes were used as the reference food in the presence of the two cinnamon types at different doses.

4.5.2 RAG and SAG values of Englyst cornflakes in the presence of cinnamon samples and acarbose at different concentrations

When 32 mg (1.28 mg/ml) of C. cassia powder was added to cornflakes in the digestive starch model, the RAG value of the cornflakes was slightly reduced. However, when
the dose of *C. cassia* was increased to 64 mg, the RAG value reduced significantly by 9.1 %. In addition, the doses of 96 mg, 128 mg, and 160 mg continued to decrease the RAG value of the cornflakes significantly by 11.51 %, 12.69 % and 16.85 %, respectively, whereas the SAG values increased significantly at the doses of 96 mg, 128 mg and 160 mg of *C. cassia* by 5.37 %, 7.84 % and 12.7 %, respectively. Similarly, *C. zeylanicum* reduced the RAG value of the cornflakes, with the same pattern for the increasing doses of *C. zeylanicum*. Although there was a trend for an increased SAG value of cornflakes in the presence of *C. zeylanicum*, this did not achieve statistical significance. The results show that cinnamon inhibits starch hydrolysis by reducing the RAG value of cornflakes, and this effect might by due to cinnamon polyphenols. The results suggest that cinnamon polyphenols interfere with the enzymes in the Englyst method, which include α-amylase and α-glycosidase, therefore cinnamon polyphenols might be able to inhibit the activity of α-amylase and α-glycosidase *in vivo*. Consequently, cinnamon polyphenols might slow glucose release from starch-based foods, in this case cornflakes, in the first 20 min (G20, RAG), and so most of it is released within 20 to 120 min (G120, SAG).

In recent years, numerous studies have focussed on the effects of phenolics on starch digestion at the *in vitro* level. Beejmohun et al. (2014) confirm that 25 µg/ml of *C. zeylanicum* alcoholic extract (100 mg/ml) inhibited 50 % of the pancreatic α-amylase activity, compared with 18 µg/ml of acarbose. Their study employed a different approach as it used 50 % ethanol to extract the cinnamon, whereas our study used cinnamon powder and cinnamon water extracts at different concentrations. In our study the *C. zeylanicum* water extracts (1 g/ml) at 12.8, 25.5 and 51.2 µg/ml, which were low concentrations compared with cinnamon powder, did not show significant inhibition of α-amylase and α-glycosidase activity. This might be due to the fact that water is not effective in extracting polyphenols, and the total phenol contents was low (see section, 3.4.1.2). However, in our study the acarbose significantly inhibited α-amylase and α-glycosidase activity, as expected. This was shown by the RAG value of the cornflakes being significantly reduced by 26.5 %, 21 % and 14.7 % at concentrations of 12.8, 25.5 and 51.2 µg/ml, respectively. Ranilla et al. (2010) highlighted that water *C. zeylanicum* extract (50 mg/ml) inhibited α-amylase and α-glycosidase activity. The Ranilla et al. (2010) method used α-amylase and α-glycosidase assays (described in Worthington Biochemical Corp., 1993 a; Worthington Biochemical Corp., 1993 b). In their study with
C. zeylanicum extracts at 1.25 mg/ml and 2.5 mg/ml inhibited α-amylase activity by 72 % and 77 %, respectively. These cinnamon concentrations were similar to the concentrations of cinnamon powder that were used in our study, which were of 32 mg (1.28 mg/ml) and 64 mg (2.56 mg/ml), which reduced RAG values of the cornflakes to be 75.35 % and 71.5 %, respectively. Ranilla et al. (2010) also investigated the effects of C. zeylanicum extract on α-glycosidase activity; they found that cinnamon concentrations of 12.5 and 25 mg/ml inhibited 95 % and 100 % of α-glycosidase activity, respectively. Thus, carbohydrate digestion was reduced by cinnamon and could be an approach to maintain and control postprandial blood glucose levels.

Although, the studies carried out by Ranilla et al. (2010) and Beejmohun et al. (2014) used different approaches to extract C. zeylanicum as well as the enzymatic assays employed compared with our study, it seems they agree that cinnamon has the potential to inhibit the activity of α-amylase and α-glycosidase, which are essential in the enzyme mixture of the Englyst method. However, these studies did not use a positive control to compare their outcomes, whereas in this study, we used acarbose as a positive control at different concentrations (12.8, 25.6 and 51.2 µg/ml), which significantly reduced the RAG and SAG values of the cornflakes. This effect of acarbose on the RAG values confirmed its ability to inhibit α-amylase and α-glycosidase activity. Our study is the first study to investigate the effects of two types of cinnamon (C. zeylanicum and C. cassia) on starch digestion at different doses, and is the first study to investigate the effects of C. cassia on enzymes activity. In a recent review on the interactions between phenolic compounds and enzymes, it was emphasised that medicinal plants rich in phenolic compounds, such as tannin, decrease starch digestion (Zhu, 2015). Studies have suggested that complex tannins, such as proanthocyanidins, could bind to the digestive enzymes, thereby inhibiting starch hydrolysis (Barrett et al., 2013). This has also been shown by Mkandawire et al. (2013) who confirmed that higher molecular weight tannins cause a greater inhibition of α-amylase than the lower molecular weight tannins.

Type-A procyanidins, which are condensed tannins, have been identified in C. cassia, and these might be the reason for cinnamon’s ability to work as a potent natural α-amylase inhibitor (Anderson et al., 2004). This has also been shown by a study which confirmed that procyanidins at different doses from cocoa extracts inhibited pancreatic α-amylase activity in vitro (Zhu, 2015).
The results in chapter 3 demonstrated that *C. cassia* had higher concentrations of total polyphenols (105.6 mg/g equivalent to Gallic acid) than *C. zeylanicum* (39.6 mg/g equivalent to Gallic acid). In addition, *C. cassia* was richer in proanthocyanidins (41.5 mg/g equivalent to procyanidin-A2) compared to *C. zeylanicum* (11.4 mg/g equivalent to procyanidin-A2). Furthermore, the LC-MS results for each cinnamon variety showed that *C. cassia* had two types of the B-type dimer, while *C. zeylanicum* had only one B-type dimer of the proanthocyanidins, and *C. cassia* had two types of A-type trimer, whereas *C. zeylanicum* only having one A-type trimer. Two major A-type proanthocyanidin tetramers were present in *C. Cassia* and *C. zeylanicum*, but at different concentrations in each. These results indicate that they could have potentially different responses due to their different polyphenol content. However, polyphenols might impact upon carbohydrate metabolism at many levels via reducing the glycaemic response, and by inhibition of carbohydrate digestion, and glucose absorption (Hanhineva et al., 2010).

Studies have suggested that slowing the starch digestion via phenolic components might increase satiety by modulating glucose levels after carbohydrate rich meals (Barrett et al., 2013). The effects of polyphenolic foods on postprandial blood glucose responses have been investigated in human studies; for instance, strawberry extracts and green tea polyphenols have been shown to inhibit α-amylase and reduce postprandial hyperglycaemia (Lo Piparo et al., 2008). Furthermore, consuming 6 g of cinnamon with rice pudding had the effect of reducing the glycaemic responses significantly ($P < 0.05$) in 14 healthy participants (Hanhineva et al., 2010). A further study of the interactions between phenolic compounds and starch-based foods highlighted that a combination of black tea polyphenols and starch (maize starch) could be used to manipulate postprandial glycaemic responses (Liu et al., 2011; Zhu, 2015). Previous studies have shown that RAG values are positively correlated with GI ($P = 0.01$), while SAG values are negatively correlated with GI ($P = 0.04$) (Garsetti et al., 2005).

**4.6 Conclusions**

Overall, both types of cinnamon powder (*C. cassia* and *C. zeylanicum*) appeared to have the ability to reduce RAG values and increase SAG values for cornflakes. This may be due to the polyphenolic compounds which might inhibit the carbohydrate
digestive enzymes. However, *C. cassia* significantly increased the SAG value compared with *C. zeylanicum* at doses of 96 mg, 128 mg and 160 mg. This may be due to the concentration of polyphenolic compounds in *C. cassia* which were higher compared to *C. zeylanicum*. Acarbose is a drug that inhibits α-glucosidase and α-amylase activity and is used for treating type 2 diabetes. The RAG and SAG values of cornflakes in the presence of acarbose were used as controls to compare with the RAG and SAG values of cornflakes in the presence of both cinnamon types at different concentrations. These results show that *C. cassia* acts better than *C. zeylanicum* to reduced RAG value via potentially inhibiting the α-glucosidase and α-amylase activity, therefore the next chapter investigated the effect of *C. cassia* on glucose response *in vivo*. 
Assessment of the effect of a *Cinnamomum cassia* supplement on the glycaemic index of a starch rich food

5.1 Introduction

Cinnamon has a long history of use as an herbal medicine, and it has been suggested its health benefits are due to the action of polyphenols (Hamidpour et al., 2015). According to the analytical results (Chapter 3), *C. cassia* had high concentrations of phenolic compounds, tannins and in particular proanthocyanidins compared with *C. zeylanicum*. Moreover, *C. cassia* had a higher antioxidant activity compared with *C. zeylanicum*, which was also evident in water *C. cassia* extracts.

In addition, *C. cassia* was able to influence carbohydrate hydrolysis *in vitro*; the hydrolysis model showed that it lowered the rapidly available glucose (RAG) value in a dose dependent manner (Chapter 4). A low RAG value could be used as an indicator of glycaemic index (GI) and therefore cinnamon may be able to reduce glycaemic response.

The GI approach classifies starch rich foods according to their physiological effect on the blood glucose level; it is defined as the incremental area under the two-hour blood glucose response curve for a test food containing 50 g or 25 g of available carbohydrate, expressed as the percentage of the response to the same amount carbohydrate from a standard food (white bread or glucose solution) taken by the same subject (Wolever et al., 1991; Jenkins et al., 1981; Brouns et al., 2005). Based on the GI value, foods can be divided into three groups: low GI foods (GI ≤ 55) are digested and absorbed slowly and lead to a lower glycaemic response, high GI foods (GI ≥ 70) are quickly digested and absorbed and cause an increased glycaemic response, and medium GI foods are those that have a GI of greater than 55 and less than 70 (Foster-Powell et al., 2002).

The American Diabetes Association (2015) highlighted the importance and benefits of knowing the GI values of foods, as they can play an important role in terms of controlling glucose levels in diabetes. Some studies have shown there was a possible
health benefit of low GI in CVD and controlling appetite, while in obesity the benefits have been debated (Brouns et al., 2005). There is evidence to show that a high GI diet stimulates a greater level of insulin secretion than a low GI diet, which leads to hyperinsulinaemia, insulin resistance and increased beta cell demand (Jenkins et al., 1987; Miller, 1994; Ludwig, 2002). The reduction of blood glucose fluctuations throughout the day is the main benefit of consuming low GI foods, which is particularly useful for diabetic and pre-diabetic people who need to control their glucose levels (Jenkins et al., 1992; Ceriello, 1998).

Studies have shown that the glycaemic response is highly correlated with the glucose released from starchy foods when they are digested with pancreatic and brush-border enzymes *in vitro* (Englyst et al., 2003). Therefore, we investigated the glucose released from a starch-rich food (cornflakes) *in vitro* (Chapter 4), and this current chapter investigates the effect of *C. cassia* on the glycaemic response of cornflakes.

Within plants there are many compounds that can affect their rate of digestion and provide medicinal properties, for example the anti-nutrients (Greiner and Konietzny 2006). These are compounds present in grains, legumes and nuts, and include lectins, tannins, polyphenols and phytic acid. All of these compounds can bind to and interfere with nutrients affecting their absorption (Greiner and Konietzny, 2006; Alonso et al., 1999).

There is evidence to suggest that polyphenols may have the ability to reduce the glycaemic response to carbohydrate-rich foods. This effect may be due to the polyphenolic compounds inhibiting some of the digestive enzymes in the gut lumen, such as amylase, leading to reduced starch digestibility (Alonso et al., 1999; Siddhuraju et al., 2005). This has also been shown by the fact that the total phenol content in food is negatively correlated with GI in both healthy and diabetic patients (Williamson, 2013). The review by Williamson (2013) highlighted the different potential mechanisms of how polyphenols reduce GI, such as inhibiting digestive enzymes (α-amylases and α-glucosidases), or glucose transport. In the standard GI test protocol, blood samples are taken at 15, 30, 45, 60, 90 and 120 minutes after the reference, either bread or glucose solution, and also after the test food. The glucose blood sample results are used to plot the area under the curve, and the results obtained after consuming test meals are compared to those obtained after consumption of the
reference food (Jenkins et al., 1981). In the presence of compounds such as polyphenols, the area under the curve may be decreased and therefore the GI is lower (Williamson, 2013). Therefore, this study assessed glycaemic index in the present of C. cassia supplements, which are rich in polyphenols. C. cassia was chosen as there were clear benefits observed on reducing RAG and increasing SAG in vitro.

5.2 Study aims

The aims of this study are:

- To determine the GI of cornflakes in the presence of 1 g C. cassia supplement.
- To determine the GI of cornflakes in the presence of a placebo.
- To evaluate the glycaemic responses of healthy participants after the ingestion of 1 g of C. cassia supplement or the placebo.
- To compare the GI of cornflakes in the presence of 1 g of C. cassia supplement or the placebo, with a control (glucose drink).

5.3 Methods

5.3.1 Subjects

A randomised blind crossover design was used with the control glucose drink and the cornflakes, combined with either the cinnamon supplement or the placebo, in a random order on separate occasions. The study protocol was standardised using the FAO/WHO guidelines (FAO/WHO, 1997) for GI testing. The study design received ethical approval from the University of Surrey Ethics Committee (EC/2011/145/FHMS) (Appendix 4), and ten healthy volunteers were recruited from the postgraduate student and staff population at the University of Surrey, after information regarding the study was distributed via e-mails and posters. The weight, height and fasting blood glucose level of each participant was measured at baseline. Participants were interviewed before the study commenced using a screening questionnaire (Appendix 5) to assess their suitability for participation. The participants were given a participant information sheet (Appendix 6). Participants were deemed able to comply with the study protocol and gave their informed consent (Appendix 7).
5.3.2 Test food and capsules

Kellogg’s® cornflakes were used as the test food, the batch number of the cornflakes was K115176039 and they were purchased from ASDA. A 30 g portion of Kellogg’s® cornflakes, which contained 25 g of available carbohydrate, was accurately weighed on a digital balance in the kitchen of the Clinical Investigation Unit (CIU) at the University of Surrey. This was prepared in advance the night before the study and was stored in an air-tight container.

The cinnamon bark (*Cinnamomum cassia*) supplement (500 mg) was supplied by NBTY Inc, and was purchased from Puritan’s Pride Ltd. In order to use the cinnamon in a capsule form, whereby the participants would not know which capsule was the placebo and which contained cinnamon, the cinnamon dose was transferred to new brown capsules. These Vcaps® capsules were made from hydroxypropylmethyl cellulose and manufactured by Capsugel in Bornem, Belgium. The capsules were presented either empty for participants consuming the placebo, or filled with 500 mg cinnamon for the cinnamon trial. The filled capsules were prepared at Stickland pharmacy in London using their accurate balance and devices to transfer the cinnamon powder from the manufactured capsule to the new capsule (Vcaps® capsules). The amount of cinnamon in each new capsule was 500 mg (±10 mg). The solubility of Vcaps® capsules was tested (section 5.3.7), and they were found to take 15 minutes to dissolve.

5.3.3 Study design

The study protocol was standardised using the FAO/WHO guidelines (FAO/WHO, 1997) and the recommendations of Brouns et al. (2005). To determine GI, each of the participants consumed 25 g of available carbohydrate in the form of cornflakes or the reference food (25 g of anhydrous glucose dissolved in water) after an overnight fast of 12-13 hours. Finger-prick capillary blood samples were taken after the overnight fast (-15 minutes), and the participants were then asked to ingest either the cinnamon or placebo capsules. Another blood sample was collected 15 minutes later (designated time 0 minutes). Further samples were taken at 15, 30, 45, 60, 90 and 120 minutes after consumption of the cornflakes. For the control study (response to 25 g glucose in 250 ml water), blood samples were collected at 0, 15, 30, 45, 60, 90 and 120 minutes. Capillary blood samples were collected in 300 µl tubes with fluoride oxalate
(Microvette, Sarstedt, Leicester, UK). Unistik 3 Extra (Owen Mumford Ltd., Oxford, UK) single use lancets were used to obtain the blood samples.

On two separate occasions the cinnamon supplement capsules (n = 2, total 1000 mg cinnamon) were ingested with 100 ml of water 15 minutes (-15 minutes) prior to the test food (cornflakes). Then 15 mins later, a portion of cornflakes containing 25 g of available carbohydrate was served to the participants with 150 ml of water (ingesting the cornflakes within 10 min). On two other separate and randomised occasions the participants ingested the placebo capsules (n = 2) with 100 ml water 15 minutes prior to the portion of cornflakes. On three randomised occasions the participants consumed the control reference food, which was a glucose drink made from 25 g of glucose in 250 ml of water (Sigma, Cat. No. G 8270).

5.3.4 Blood sample collection

Capillary finger prick blood samples were collected in 300 µl Microvette tubes coated with fluoride oxalate, and were immediately centrifuged at 2000 g for 10 minutes, at 4 °C. The centrifuged plasma was transferred to separate 300 µl plain plastic Microvette tubes. The tubes were then kept in the freezer at -20 °C until analysis.

5.3.5 Glucose measurement

An automatic analyser (YSI 2300 STAT plus, Yellow Springs, Analytical Technologies, YSI Ltd., Fleet, UK) was used for determining plasma glucose concentration. Three quality controls were used to determine the intra- and inter-assay coefficients of variation (Section 2.2.8.4.2).

5.3.6 Calculation and statistical analyses

The incremental areas under the glucose curve (iAUC) for the reference glucose drink, cornflakes with cinnamon capsules, and cornflakes with placebo, were calculated according to the method recommended by the FAO/WHO (1997). The GI values for cornflakes with cinnamon capsules, cinnamon group, and cornflakes with the placebo, placebo group, were calculated as follows (source Wolever et al., 1991; Brouns et al., 2005):
The GI values of cornflakes with either the cinnamon capsules or the placebo were calculated as an average value obtained from 10 subjects.

Results were checked for normality using the Kolmogorov-Smirnov test (K-S test) and were expressed as the mean ± one standard error of the mean (SEM). For the human study, a two factor (treatment and time) repeated measures analysis of variance (ANOVA) was used to analyse the difference in the mean glucose level at each time point for the two groups (cinnamon and placebo), and their standard glucose response across the time course. In addition, a single factor (treatment) repeated measures ANOVA was used to analyse the differences in iAUC for glucose (control) and the test food (SPSS 22 for Windows).

5.3.7 Vcaps® capsules, hydroxypropylmethyl cellulose, solubility

In order to identify the time taken for the capsules to dissolve, they were added to water. To simulate the acidity and temperature of the stomach, the capsules were added to 250 ml MilliQ water, with the pH adjusted to 2.1 by the addition of 0.1 M of HCL, and heated in a water bath at 37 °C. The time that each capsule took to dissolve was recorded, and the average time taken for the capsules to dissolve was 15 minutes. Therefore, the cinnamon capsules were given to the participants 15 minutes prior to the portion of cornflakes.

5.4 Results

The characteristics of participants (Table 5.1) were investigated at the start of the study. They had a mean age of 33 years, were in the overweight category according to the average BMI score (26.7 kg/m²), and they had normal fasting blood glucose levels. The 10 individuals recruited consisted of 4 females and 6 males, mean age 33.3 years, age range 21 – 51 year.
Table 5.1: The characteristics of the subjects.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>33.3 ± 3.5</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>79.8 ± 5.9</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.7 ± 0.02</td>
</tr>
<tr>
<td>BMI (kg/m^2)</td>
<td>26.7 ± 1.2</td>
</tr>
<tr>
<td>Fasting blood glucose (mmol/l)</td>
<td>5.1 ± 0.05</td>
</tr>
</tbody>
</table>

Result expressed as Mean ± SEM (n=10 subjects)

The incremental area under the curve (iAUC) for glucose was calculated for the reference (glucose drink), cornflakes with cinnamon capsules, and cornflakes with placebo (Figure 5.1). The iAUC for the cornflakes with cinnamon supplement and placebo were 146 ± 36 and 146 ± 33, respectively \((P = 1.00)\), which meant the GI value of the cornflakes with placebo and with cinnamon capsules were not significantly different \((P = 0.970)\), as the GI value for the cornflakes ingested with the cinnamon supplement was 99 ± 12, while the GI for the cornflakes with placebo was 98.9 ± 9. Overall, no significant differences were seen in glucose response for the different times, or in the incremental area under the curve between the \(C. cassia\) supplement and the placebo, but there were differences between the control glucose drink and the cornflakes (Figure 5.1). Furthermore, there was no significant difference between the fasting plasma glucose (FPG) levels for cinnamon and the placebo \((P = 0.84)\) (Figure 5.2), which is as expected.

The peak glucose level (Figure 5.1) for the standard glucose drink occurred at 30 minutes, whereas the peak glucose level for the cornflakes with cinnamon and the placebo occurred at 45 minutes. This shift in the peak glucose level may be due to the type of carbohydrate and the other components in the cornflakes, which caused the glucose to be released slower than the control solution. At 30 minutes, the peak glucose level for the standard glucose drink was higher than the peak glucose levels for both cornflake groups. For the cornflakes, with both cinnamon and the placebo, the glucose levels declined slowly over the next 45 minutes and then gradually dropped to the baseline. On the other hand, the glucose level for the standard glucose drink dropped rapidly to below the baseline by 90 minutes, almost a 0.30 mmol/l reduction, and the level then increased towards the baseline during the final 30 minutes. This
shows how the control and the treatments produce different results over time due to the composition of the test material. The results (Figure 5.2) also show that 1 g of cinnamon supplement produced no effect on fasting glucose. Moreover, the fasting glucose levels for the placebo were identical to the cinnamon group.

Figure 5.1: Plasma incremental glucose responses during the 2 hours following the reference glucose, and cornflakes with cinnamon supplement or placebo (results presented as mean (n = 10) ± SEM).
Chapter 5

Figure 5.2: Fasting plasma glucose levels (results presented as mean (n = 10) ± SEM).

5.5 Discussion

This human study examined the effects of a *C. cassia* supplement on the GI of a starch rich food (Kellogg's® cornflakes). This brand of cereal was used in the human study because it is high in starch and is commonly consumed at breakfast time in the UK and other countries. Cornflakes are also used frequently in GI studies.

The *C. cassia* supplement was used in this study due to its effectiveness in terms of reducing RAG in vitro. Englyst et al. (1996) confirmed that the RAG values for a range of carbohydrate rich foods were found to correlate strongly with their GI values *in vivo*. The GI of cornflakes in the presence of 1 g of cinnamon and a placebo were found to be 99 ± 12 and 98.9 ± 9, respectively. This value was slightly higher than the GI value of cornflakes in the Harvard list of GI and glycaemic load for over 100 foods (which lists the GI of cornflakes as 93), however Wolever and Bolognesi (1996) also showed the GI value of cornflakes to be 99, which agrees with our study data. It is evident that the GI of cornflakes can vary, for example, Atkinson et al. (2008) published an international table listing the GI for 2480 foods. This table referenced five studies that determined the GI of Kellogg's® cornflakes, which ranged from 72 ± 16 to 132 ± 33. Four of these studies used a glucose drink as a reference and three of them were focussed around a health topic. Overall the average GI from these five studies was 95 ± 10, which is similar to the outcome of this study. The variability of GI in the same food
depends on the reference food used, the health state of the subjects, and the methodology. Furthermore, some manufacturers produce the same food and brand but with differences; for instance, Kellogg’s Special K and All-Bran have different formulations in North America, Europe, and Australia (Atkinson et al., 2008). Therefore, some variability in the GI value is expected.

This study showed that ingesting 1 g of cinnamon supplement before a portion of cornflakes was not enough to significantly reduce the glucose response when compared to a placebo. Some acute studies involving cinnamon support the results of this study (Table 5.2) as they have similar results. The first study conducted by Hlebowicz et al. (2009) did not observe a change in glucose level after consuming *C. cassia*, which added as either 1 g or 3 g to 300 g of rice pudding. They found that the dose of cinnamon had no effect on blood glucose in healthy participants, however, the higher dose (3 g) of *C. cassia* did affect postprandial plasma insulin levels, which shows the cinnamon did have some effect. Interestingly, adding a larger dose of 6 g of cinnamon to the rice pudding, significantly lowered the postprandial glucose response and delayed gastric emptying (Hlebowicz et al., 2007), which suggests a possible reason for the lack of effect in our study was due to the small dose used. Importantly, neither of these were blinded studies, and the participants were aware that they were consuming cinnamon with rice pudding. Also, the cinnamon dose was administered together with the rice pudding. However, in our study the participants consumed the cinnamon in capsule form 15 minutes prior to the cornflakes, which could have had an effect on its activity. Other research supporting the current findings showed that ingesting 3 g of *C. zeylanicum* in capsule form, 1.5 g directly before and 1.5 g after a test meal comprising 42 g carbohydrate, 46 g fat and 13 g protein, did not change blood glucose concentrations in healthy subjects (Markey et al., 2011). Although they used a high fat test meal (a factor which can have an effect on GI value), the ingestion of 3 g of *C. zeylanicum* supplement with this meal did not delay gastric emptying or effect glucose level (Markey et al., 2011).
Table 5.2: Summary of previous acute studies on the effect of cinnamon on glucose control.

<table>
<thead>
<tr>
<th>Reference / Year</th>
<th>Number and Condition of Subjects</th>
<th>Cinnamon Class / Dose / Duration</th>
<th>Method of Consuming Cinnamon</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solomon and Blannin (2007)</td>
<td>7 healthy male subjects</td>
<td><em>Cinnamomum cassia</em> / 5 g of cinnamon was ingested 12 h prior OGTT, OR 5 g of cinnamon was ingested with OGTT</td>
<td>Cinnamon was presented in capsule form</td>
<td>Plasma glucose response decreased significantly and insulin sensitivity was higher than control.</td>
</tr>
<tr>
<td>Hlebowicz et al. (2007)</td>
<td>14 healthy subjects</td>
<td><em>Cinnamomum cassia</em> / 6 g / short term duration</td>
<td>Cinnamon dose was added to 300 g of cooked rice pudding</td>
<td>Cinnamon with rice pudding reduced postprandial blood glucose and delayed gastric emptying.</td>
</tr>
<tr>
<td>Hlebowicz et al. (2009)</td>
<td>15 healthy subjects</td>
<td><em>Cinnamomum cassia</em> / 1 g and 3 g / short term duration</td>
<td>Cinnamon dose was added to 300 g of rice pudding</td>
<td>No significant effect on glucose or gastric emptying rate for either dose of cinnamon. However, 3 g dose of cinnamon reduced serum insulin response.</td>
</tr>
<tr>
<td>Markey et al. (2011)</td>
<td>9 healthy subjects</td>
<td><em>Cinnamomum zeylanicum</em> / 3 g / short term duration</td>
<td>Cinnamon was presented in capsule form</td>
<td>No effect on glucose concentration, postprandial or gastric emptying parameters.</td>
</tr>
<tr>
<td>Wickenberg et al. (2012)</td>
<td>10 subjects with impaired glucose tolerance</td>
<td><em>Cinnamomum zeylanicum</em> / 6 g with OGTT (short term duration)</td>
<td>Cinnamon was presented in capsule form</td>
<td>No significant effect on plasma glucose or insulin level.</td>
</tr>
<tr>
<td>Magistrelli and Chezem (2012)</td>
<td>30 subjects (15 normal weight, 15 obese)</td>
<td><em>Cinnamomum cassia</em> / 6 g / short term duration/</td>
<td>Cinnamon dose was added to cooked cream of wheat instant farina cereal</td>
<td>Significantly reduced glycaemic response.</td>
</tr>
</tbody>
</table>
The results in the study by Markey et al. (2011) showed that cinnamon had no effect on glucose level, which could be explained as follows; although they used 3 g of cinnamon, 1.5 g before the test meal, which consisted of 42 g of carbohydrate, 46 g of fat and 13 g of protein, and 1.5 g after the test meal, this was not enough to have an effect, possibly because the high fat content of the meal (46 g fat) affected glucose absorption from the small intestine (Normand et al., 2001). Furthermore, it might be because the dose of cinnamon relative to the carbohydrate in the test meal (carbohydrate/cinnamon ratio) was low ratio (14:1) compared to other studies, where the carbohydrate/cinnamon ratio was (15:1), which found there was a reduction in glucose levels (Solomon and Blannin, 2007). In the Solomon and Blannin (2007) study the participants ingested 5 g of *C. cassia* supplement with, or 12 hours prior to, an oral glucose tolerance test OGTT (75 g bolus of dextrose in 300 ml water), which resulted in a significantly reduced plasma glucose response. The dose of *C. cassia* was also higher than ours (5 g), which might be enough to inhibit glucose transport resulting in a reduced glucose response in healthy subjects. They also employed a different approach, where the cinnamon supplement was consumed 12 hours prior to the OGTT to preload the subjects with a large dose of cinnamon. This approach might allow time for the cinnamon polyphenols, such as procyanidin, to be present in the gut, leading to an effect on glucose levels and reduce the glucose response. Polyphenols can contribute to multiple mechanisms in the gut lumen, which include improving fasting glucose, inhibiting glucose transport, inhibiting digestion enzymes and improving insulin sensitivity (Williamson, 2013).

Some studies found that cinnamon had no effect on glucose response, although they used high doses of cinnamon, for example Wickenberg et al. (2012) found that ingesting 6 g of *C. zeylanicum* (14 capsules) with an oral glucose dose (75 g) did not have a significant effect on the glucose response, insulin level or GI. This ineffective result might due to the type of cinnamon used, as *C. zeylanicum* has been shown to have a lower concentration of polyphenols (section 3.4.1.1). In contrast, when 6 g *C. cassia* was ground into 74 g of cream wheat instant farina cereal (containing 50 g available carbohydrate), the resulting blood glucose concentration was significantly reduced compared to the same meal without *C. cassia* (Magistrelli and Chezem, 2012). This effect could again be due to the cinnamon type, which was *C. cassia* which is rich in polyphenols. Studies show that the polyphenols in cinnamon might inhibit α-amylase
due to the hydroxyl group in the polyphenols binding with α-amylase (Xiao et al., 2011),
and cinnamon may slow the absorption of carbohydrates in the small intestine (Kim et
al., 2006). Polyphenols were found to increase insulin sensitivity (Weichselbaum and
Buttriss, 2010). Anderson et al. (2004) isolated the A-type procyanidin oligomers from
C. cassia, and they found that these polyphenolic polymers enhanced the biological
activity of insulin in vitro, which may be beneficial in controlling glucose intolerance and
diabetes. Moreover, Lu et al. (2011) found that C. cassia is rich in B-type procyanidin
oligomers, which was identical to our findings (Chapter 3), as the LC-MS of the
polyphenol composition of cinnamon showed that C. cassia has two B-type dimers and
two B-type trimers, while C. zeylanicum has only one type of each procyanidin, and
both had two major A-type tetramers.

It is clear from the in vitro and in vivo studies that the type of cinnamon and size of
dose play important roles in the level of biological benefits. C. cassia has a positive
effect compared with C. zeylanicum, although this study used C. cassia, there was no
effect on glucose level, possibly because the dose (1 g) was low and therefore
insufficient to affect glucosidase, amylase activity and insulin sensitivity. Furthermore,
the carbohydrate/cinnamon ratio was (25:1), which was perhaps too low in terms of
cinnamon dose and thus did not achieve a significant effect on glucose response. In
addition, the power of the study was possibly not enough to see an effect of cinnamon
in glycaemic index. The fact that the participants were healthy individuals with a normal
glucose level may have also made it more difficult to see an effect.

5.6 Conclusions

Overall these findings showed that ingesting 1 g of C. cassia supplement prior to
consuming a high GI food (cornflakes) did not affect the glycaemic response in healthy
overweight participants, as the GI values of cornflakes with cinnamon, and cornflakes
with a placebo, were similar. No significant differences were found in glucose response
for the different time periods, nor in the incremental area under the curve for the C.
cassia supplement and the placebo. The results suggest that 1 g of cinnamon is
insufficient to affect the glycaemic response. Further research efforts, particularly long
term studies, are required to ascertain the health benefits of high doses of C. cassia
supplement.
Chapter 6

An evaluation of serum inflammatory marker levels in overweight women consuming a *Cinnamomum cassia* supplement for eight weeks

6.1 Introduction

Diabetes and the associated disorder of metabolic syndrome (MetS) are ever increasing worldwide health problems, especially due to the oxidative stress and chronic inflammation that are associated with MetS (Gyawali et al., 2015). Another world wide health problem is obesity, which can be described as a chronic low-grade inflammation, which serves as a major risk factor for hypertension, coronary artery disease, dyslipidaemias, and type 2 diabetes (Hotamisligil, 2006; Nehete et al., 2014). The inflammation is as a result of adipocytes in overweight individuals releasing inflammatory markers that may lead to diabetes (Hotamisligil, 2006). There are two types of inflammation: acute and chronic; acute inflammatory responses, by definition, generally progress over a short period of a few days, whereas chronic inflammatory responses continue over a longer time period, from months, to years, to a lifetime (Nayak et al., 2013; Fleit, 2014).

Inflammation is the biological response of the innate immune system, enabling it to protect the body against harmful stimuli such as pathogens, damaged tissues and irritation (Jungbauer and Medjakovic, 2012; Furie, 2014). In acute inflammation leukocytes, and in particular neutrophils, are recruited from the blood to the site of inflammation. This movement of leukocytes from the bloodstream to the affected site is regulated by adhesion molecules such as ICAM, P-selectin, and chemo-attractants such as MCP-1 and IL-8. The neutrophils then release pro-inflammatory cytokines such as IL-1, IL-6, tumour necrosis factor (TNF-α) and interferon gamma (IFN-γ) (Jungbauer and Medjakovic, 2012; Furie, 2014).

The level of inflammatory markers are often elevated with increasing age (Prasad et al., 2012). Studies have shown that the circulating steroid hormone concentrations are decreased with increasing age, and that may contribute to the elevation of pro-inflammatory markers (Cartiera et al., 2009). Singha et al. (2011) highlighted that numerous studies describe an increase in pro-inflammatory cytokine levels after the menopause. Moreover, women have a higher percentage of body fat than men (Blaak,
2001), and inflammatory cytokines, such as TNF-α, are secreted 7.5 times higher from the adipose tissues (Ratliff et al., 2008). Therefore, women after the menopause have an increased chance to have high levels of inflammatory markers, which were chosen for this study.

Early diagnosis of some diseases such as diabetes, hypertension and CVD, is relatively straightforward and can help to delay the progression of the disease. Liu et al. (2007) identified that high sensitivity C-reactive proteins (hs-CRP), IL-6 and the TNF-α receptor can be used as biomarkers for predicting risk of developing diabetes in healthy postmenopausal women (Liu et al., 2007). This is supported by studies that show elevated levels of cytokines may promote insulin resistance in liver, skeletal and vascular endothelia, which might lead to type 2 diabetes and CVD (Wellen and Hotamisligil, 2005).

MetS is a combination of conditions that includes high blood pressure, insulin resistance, an excess of body fat around the waist, high cholesterol and high fasting glucose concentrations (Vidigal et al., 2015). In MetS there was a linear increase in the level of hs-CRP (Gyawali and Richards, 2015), and increased levels of CRP were significantly associated with a high BMI ($P = 0.012$), triglycerides ($P = 0.001$) systolic blood pressure ($P = 0.019$) (Fredrikson et al., 2004), and also associated with insulin resistance and HDL cholesterol (Ford, 2003; Lambert et al., 2004).

Studies have shown inflammation can be decreased by many approaches including diet, exercise, cardiovascular drugs, and insulin sensitizers; a combination therapy such as lifestyle modifications and multiple drugs, might be the most effective therapy to reduce inflammation (Koh et al., 2005). There is evidence to suggest that in overweight and obese individuals, losing weight is an effective way to reduce the risk of developing chronic inflammatory disease, diabetes, and CVD, especially if combined with exercising and ingesting herbal supplements (Anderson et al., 2003). Non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin and ibuprofen are regularly used to treat a variety of inflammatory conditions. However, if they are taken over the long term, NSAIDs have side effects such as causing liver failure and high blood pressure (Gunawarden et al., 2014). Although NSAIDs have been shown to be efficacious, looking for alternative natural strategies to treat inflammatory diseases is
important such as using food and plant products containing compounds with anti-inflammatory properties, such as polyphenols (Gunawarden et al., 2014).

In recent years the use of herbal medicine has increased, and it has attracted the attention of researchers all over the world, as medicinal herbs have been reported to show antioxidant, antimicrobial and anti-inflammatory properties (Upendra et al., 2010). Approximately 50 % of approved drugs are derived from plant products, or their derivatives (Eisenberg et al., 2011). Indeed, the use of herbal products to treat and prevent disease in the USA has increased from 2.5 % in 1990 to 18 % in 2007 (Eisenberg et al., 2011). This suggests using herbal medicines could be a promising area in the treatment, and also the prevention, of some chronic diseases; however, more clinical research is needed to observe the effects of these herbal products in terms of disease progression, and changes in the modulation of biomarkers that occur in humans before wide scale use can be suggested.

There have been several mechanisms suggested to explain the anti-inflammatory action of herbal medicines, including their role in the inhibition cyclooxygenases, nitric oxide synthase and cytokines such as TNF-α (Gunawarden et al., 2014). Cinnamon (Cinnamomum cassia, family Lauraceae) has been used within many cultures as a traditional herbal medicine for the treatment a number of diseases (Ravindran et al., 2004). Studies, both in vitro and in vivo have reported that cinnamon has anti-inflammatory, anti-diabetes, anti-microbial, antioxidant, anti-tumour, anti-fungi, antiviral, blood pressure lowering, lipid lowering and immunomodulatory effects (Gruenwald et al., 2010). For example, studies have shown that C. cassia inhibited the growth of Escherichia coli (E. coli), and can therefore be considered as an anti-bacterial agent (De et al., 1999; Oussalah et al., 2006).

Some of the main processes involved in inflammatory and immune diseases are oxidative processes and free radical production. Therefore the use of antioxidants in pharmacology, such as vitamin C is widely studied (Clark, 2002). There are also many studies that have reported that cinnamon has important antioxidant properties, for example in vitro, an alcoholic extract of C. cassia showed a greater (96.30 %) inhibition of lipid peroxidation in rat liver compared, with α-tocopherol (93.74 %), indicating it is a strong antioxidant (Lin et al., 2003). Our study (chapter three) has also confirmed that cinnamon has strong antioxidant activity.
There are more studies that have shown health benefits of cinnamon, for example *in vivo* *C. cassia* extract showed antipyretic activity on virus infected mice models, and this antipyretic activity was significantly correlated with IL-1α (Kurokawa et al., 1998). There is also evidence that *C. cassia* extract inhibits the activity of cyclo-oxygenase-2 (COX-2) and nitric oxide (NO), enzymes that are induced by pro-inflammatory cytokines, and growth factors, in mouse macrophage cell line models (Hong et al., 2002).

These antioxidant properties might influence the immunomodulatory activity of cinnamon, which may affect the anti-inflammatory effect of cinnamon (Gruenwald et al., 2010). The main active compounds in *C. cassia* bark are the high levels of non-volatile compounds (mainly condensed tannins) which contain 23.2 % proanthocyanidins and 3.6 % epi-catechin monomers (Shan et al., 2007). Lee et al. (2005) and Kim et al. (2007) found that cinnamaldehydes isolated from *C. cassia* inhibited COX-2 and NOS *in vitro*, which may be responsible for the anti-inflammatory and anti-oxidant properties of cinnamon.

The efficacy of cinnamon has been documented as an anti-inflammatory in *vitro* and in animal studies, however, this has not been extensively tested in humans. Therefore, this study is going to investigate the effect of consuming *C. cassia* supplement on inflammatory markers.

### 6.2 Study aims

- To investigate the effect of consuming *C. cassia* supplements (5 g) on cytokine and growth factor levels (IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, EGF, IFN-γ, MCP-1, TNF-α, VEGF) in overweight women aged 45-70 years for eight weeks.
- To examine the effect of consuming *C. cassia* supplements (5 g) on high sensitive C-reactive proteins (hs-CRP) in overweight women aged 45-70 years for eight weeks.
- To examine the effect of consuming *C. cassia* supplements (5 g) on adhesion molecule levels (VCAM, ICAM, E-selectin, P-selectin, L-selectin).
- To examine the effect of *C. cassia* supplementation (5 g) on blood pressure in overweight women aged 45-70 years for eight weeks.
- To examine the effect of consuming *C. cassia* supplements (5 g) on anthropometric measurements in overweight women aged 45-70 years for eight weeks.

### 6.3 Methods

#### 6.3.1 Subjects

The supplementation study was a randomised controlled and single blind trial. This study design received ethical approval from the University of Surrey Ethics Committee (EC/2013/70/FHMS), (Appendix 8).

A power calculation estimated for this study was based on data of fasting glucose from previous studies, as there were no preceding studies based on the effect of cinnamon on inflammatory markers. The primary outcome variable was fasting glucose, which was shown by Khan et al. (2003) who observed a 2 mmol/L fall in fasting glucose. Based on this data, a common standard deviation (SD) of 1.0 was assumed. The effect at 90 % power required 18 individuals for the cinnamon group and 18 for the placebo group. An additional twenty-five percent was added to allow for dropout from the study, therefore the recruitment target for each group was 23 women. Participants were recruited using a poster with details of the study placed at multiple sites around the University of Surrey. This information was also emailed to all University staff. Posters (Appendix 9) were also placed in local sites relatively close to the University, including churches, train stations, bus stops, restaurants, supermarkets, health centres and hospitals in Guildford, Woking, Kingston and London. A presentation about the cinnamon study to women in a Woking Mosque was also used for recruitment where they were able to listen to and ask questions about the study.

#### 6.3.2 Screening

Participants were required to fast for a 12 hour period overnight prior to screening, and blood glucose levels were tested in the morning at the Clinical Investigation Unit (CIU), University of Surrey, using the finger prick method. Furthermore, the participants’ weight and height were taken, and they answered a screening questionnaire regarding their health and lifestyle (Appendix 10). The participants were given a participant information sheet (Appendix 11) and a consent form (Appendix 12), and if they were happy to take part they signed the informed consent form. These screening procedures
were undertaken to ensure the participants were eligible and healthy so they could be enrolled in the study.

6.3.3 Study participants

Inclusion criteria were:

- Female aged 45-70 years
- Free from medication
- Free from cinnamon allergy
- Overweight or obese (BMI ≥ 25)
- Not taking, or had taken within the last month, any supplements and/or herbal supplements
- Non-smokers
- Healthy and free from any disease
- Able and willing to comply with all study procedures

6.3.4 Study design

The study was a randomised controlled and single blind design study with subjects randomly assigned to either the cinnamon supplementation group or the control (placebo) group. The duration of supplementation was eight weeks. The study consisted of three visits to the CIU for blood samples and measurements to be taken.

A cinnamon \((C.\ cassia)\) supplement which was supplied by NBTY Inc, and purchased from Puritan’s Pride, was used in this study. These capsules were emptied and then transferred to new opaque coloured capsules \((\text{CapsulCN®})\), which were manually filled with cinnamon or corn flour, so both filled capsules appeared identical. \text{CapsulCN®} capsules were made from bovine gelatine \((\text{Halal})\). The daily dose was 5 g, as either cinnamon or placebo, which meant each participant consumed three capsules \((\text{cinnamon or placebo})\) after breakfast and three capsules \((\text{cinnamon or placebo})\) after their evening meal each day for eight weeks (Figure 6.1).

On the first study day participants attended the CIU in the early morning having consumed a standardised meal \((\text{vegetable lasagne, 400 g})\) with known macronutrient composition, the evening before followed by a 12 hour fast. This meal was given before each study day to minimise variability. Participants were also told to refrain from intense physical exercise and the consumption of alcohol for three days prior to each
study day. Volunteers also refrained from consuming caffeine containing drinks (tea/coffee/carbonated soft drinks such as Pepsi) for 24 hours before each visit.

All subjects recorded a diet diary to calculate their food intake for three days prior to each study day. Diet plan 6, Forest field software Ltd, was used to calculate the macronutrient content of their food intake during these three days, at each visit.

Fasting blood samples, blood pressure readings and anthropometric measurements were taken at each visit. On visit one only, the Peripheral Quantitative Computed Tomography (pQCT) was used to scan the wrist bones of the participants. The ionising radiation that participants were exposed to from the pQCT was at an extremely low level (0.5µ Sv).
Figure 6.1. Study design for the investigation of the effect of cinnamon over an 8-week study period.
6.3.5 Blood sample collection

Fasting blood samples were taken at week zero, week four and week eight. They were analysed for cytokines and growth factors (IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, EGF, IFN-γ, MCP-1, TNF-α, VEGF), adhesion molecules (VCAM, ICAM, E-selectin, P-selectin, L-selectin) and CRP. The blood samples were taken by trained phlebotomists at the CIU. 10 ml of a fasting blood sample was drawn by vacutainer using a safety lock blood collection set and a single use holder, into the vacutainer non additive tubes for serum collection (Becton Dickinson (BD) and Company, Oxford, UK). Blood samples were left for 25 min to clot, and then centrifuged at 2000 g for 10 min at 4 °C to obtain serum. Aliquots of serum were dispensed into appropriately labelled microcentrifuge tubes (1.5 ml) and stored at -80 °C until required for analysis.

6.3.6 High sensitivity cytokines and growth factor measurement

Biochip array technology, in combination with the Evidence Investigator (Randox Laboratories Ltd., Crumlin, UK) were used to determine multiple cytokine levels (IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, EGF, IFN-γ, MCP-1, TNF-α, VEGF) from a single sample in real time. The protein sandwich immunoassay method was used to detect the cytokines. Cytokine high sensitivity array (EV3623) with three quality controls (QC) were used. The inter-assay coefficient of variation of level 1, 2 and 3 QC for each marker was determined (Table 6.1). The different controls were as expected and this suggested the assay was sensitive enough to detect levels found in vivo.

6.3.7 Adhesion molecules measurement

The Evidence Investigator and Biochip array technology (Randox Laboratories Ltd, Crumlin, UK) were used to measure soluble VCAM, ICAM, E-selectin, P-selectin and L-selectin simultaneously from a single sample. The immunoassay method, which is based on protein sandwich, was used to detect the adhesion molecules. Adhesion Molecule array (EV3519) with three quality controls were used. The inter-assay coefficient of variation CVs % of level 1, 2 and 3 QC for each marker was determined (Table 6.1). The different controls were as expected and this suggest the assay was sensitive enough to detect levels found in vivo.
Table 6.1 The three quality controls (QCs) used to determine the serum markers in the Evidence Investigator biochip array.

<table>
<thead>
<tr>
<th>Inflammatory Markers</th>
<th>% CVs inter assay Level 1 (low)</th>
<th>% CVs inter assay Level 2 (medium)</th>
<th>% CVs inter assay Level 3 (high)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1α</td>
<td>3.66</td>
<td>3.28</td>
<td>2.64</td>
</tr>
<tr>
<td>IL-1β</td>
<td>3.45</td>
<td>0.32</td>
<td>3.47</td>
</tr>
<tr>
<td>IL-2</td>
<td>2.54</td>
<td>4.09</td>
<td>6.98</td>
</tr>
<tr>
<td>IL-4</td>
<td>4.53</td>
<td>9.65</td>
<td>7.08</td>
</tr>
<tr>
<td>IL-6</td>
<td>6.84</td>
<td>7.05</td>
<td>10.71</td>
</tr>
<tr>
<td>IL-8</td>
<td>4.40</td>
<td>4.20</td>
<td>3.85</td>
</tr>
<tr>
<td>IL-10</td>
<td>1.97</td>
<td>4.98</td>
<td>7.02</td>
</tr>
<tr>
<td>EGF</td>
<td>8.10</td>
<td>4.29</td>
<td>3.54</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>5.07</td>
<td>8.35</td>
<td>8.10</td>
</tr>
<tr>
<td>MCP-1</td>
<td>2.84</td>
<td>3.53</td>
<td>2.15</td>
</tr>
<tr>
<td>TNF-α</td>
<td>6.41</td>
<td>4.08</td>
<td>6.45</td>
</tr>
<tr>
<td>VEGF</td>
<td>0.04</td>
<td>3.48</td>
<td>16.79</td>
</tr>
<tr>
<td>VCAM</td>
<td>6.01</td>
<td>16.26</td>
<td>5.69</td>
</tr>
<tr>
<td>ICAM</td>
<td>8.93</td>
<td>5.99</td>
<td>0.26</td>
</tr>
<tr>
<td>E-selectin</td>
<td>10.68</td>
<td>7.68</td>
<td>10.57</td>
</tr>
<tr>
<td>P-selectin</td>
<td>3.08</td>
<td>3.78</td>
<td>2.59</td>
</tr>
<tr>
<td>L-selectin</td>
<td>10.80</td>
<td>5.29</td>
<td>0.38</td>
</tr>
</tbody>
</table>
6.3.8 CRP

An enzymatic automated colorimetric method using the ILab 650 analyser (Instrumentation Laboratory, UK) was used to measure serum CRP concentrations. A High Sensitivity C-reactive protein (hs-CRP) kit (CP3885) along with two quality control (QC) samples (Randox Laboratories Ltd., Crumlin, UK) were used. Inter-assay precision gave CVs of 8.65 % and 7.85 % for both level 1 (low) and level 2 (high) QCs, respectively. The QCs were used to make sure about the efficient use of machines and to check the variation during running the samples.

6.3.9 Statistical analyses

Statistical analyses were performed using SPSS statistical version 22 (SPSS for Windows, Chicago, USA). The data was checked for normality using Kolmogorov-Smirnov analyses and checked for both the normal and de-trended normal probability plots. The outcomes were expressed as a mean ± standard error of the mean (SEM). A repeated measures two-way ANOVA was used to study the change in inflammatory markers. An unpaired t-test was used to compare the different variables between the groups at baseline. In addition, a paired t-test was used to compare the changes at weeks four and eight with the baseline. The Pearson correlation coefficient was used to examine the relationship between all the variables. All plots presented were plotted using GraphPad Prism version 6 for Windows (San Diego, CA, USA). Each plot based on the corresponding p-value for cinnamon treatment (T), duration time (D) and interaction between treatment and time. Correlation coefficients were used to examine the relationship between changes in the different variables. Statistical significant was defined as $P < 0.05$.

6.4 Results

6.4.1 Patient recruitment

The target number of individuals required for each group in the study was not attained. Although the investigator used many different ways (section 6.3.1) to advertise the study at the University of Surrey, as well as in public areas around Guildford and Woking, and London, the number of the participants was lower than hoped for. The time of the year, which was during the summer holidays, may have had an effect on recruitment. Also, the age criterion was limited to the middle-aged (45-70 years)
healthy women. The total number of participants enrolled in the study was 16. Moreover, on analysis of the data it was evident that one of the participants as an extreme outlier; her BMI was over 40 and this was more than 2 SDs from the mean of the rest of the population. Therefore, the number of participants used to run the statistical analysis was 15, which was split into eight participants in the cinnamon group and seven participants in the placebo group (Table 6.2).

### 6.4.2 Baseline characteristics

The anthropometric and clinical characteristics of subjects at the baseline for both groups were measured (Table 6.2). The mean age, height, weight, BMI, waist circumference (WC) and hip circumference (HC) of the participants did not differ significantly between the cinnamon and placebo groups. In terms of blood pressure, there was no significant difference in diastolic blood pressure, however, there was a significant difference in systolic blood pressure between the two groups. The systolic blood pressure in the cinnamon group was higher (119.88 mm Hg) than control (108 mm Hg) at the baseline \( (P = 0.03) \). In bone trabecular density mg/cm\(^3\) (BTrbDen1mgpercm3) there was no significant different between the groups. The BTrbDen1mgpercm3 for both groups was in the normal range according to the literature (Grampp et al., 1997).
Table 6.2 The characteristics of the study participants at the baseline. *significant difference \( P < 0.05. \)

<table>
<thead>
<tr>
<th>Demographic</th>
<th>Cinnamon (n = 8) Mean ± SEM</th>
<th>Placebo (n = 7) Mean ± SEM</th>
<th>Unpaired t-test p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (m)</td>
<td>1.61 ± 0.01</td>
<td>1.65 ± 0.02</td>
<td>0.13</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>76.75 ± 2.7</td>
<td>77.29 ± 3.4</td>
<td>0.90</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.46 ± 1.0</td>
<td>28.34 ± 1.3</td>
<td>0.49</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>94.50 ± 2.2</td>
<td>89.21 ± 2.2</td>
<td>0.11</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>106.94 ± 2.3</td>
<td>108.00 ± 2.1</td>
<td>0.74</td>
</tr>
<tr>
<td>Systolic Blood Pressure (mm Hg)</td>
<td>119.88 ± 3.1</td>
<td>108.00 ± 3.5</td>
<td>0.03*</td>
</tr>
<tr>
<td>Diastolic Blood Pressure (mm Hg)</td>
<td>83.00 ± 3.2</td>
<td>74.71 ± 3.5</td>
<td>0.10</td>
</tr>
<tr>
<td>Heart Rate (BPM)</td>
<td>67.50 ± 2.2</td>
<td>72.37 ± 3.9</td>
<td>0.28</td>
</tr>
<tr>
<td>Age (Years)</td>
<td>56 ± 1.3</td>
<td>53 ± 2.7</td>
<td>0.22</td>
</tr>
<tr>
<td>Bone Trabecular Density mg/cm³ (BTrbDen1mgpercm³)</td>
<td>182.70 ± 14.39</td>
<td>167.42 ± 11.74</td>
<td>0.434</td>
</tr>
</tbody>
</table>

6.4.3 Changes in anthropometric indices and blood pressure after supplements

There were no significant differences in body weight, height, WC, HC and BMI between the two groups at baseline (Table 6.2), however, after four weeks of cinnamon supplementation (Table 6.3), there was a significant decrease in body weight for the cinnamon group \( (P < 0.001) \), and the WC and BMI also significantly decreased \( (P = 0.04 \) and \( P < 0.001 \) respectively), however no changes in these parameters occurred in the control group, which suggests the difference is due to the cinnamon supplementation having an effect.

After 8 weeks (Table 6.3) there were further significant reductions for the cinnamon group; body weight decreased significantly \( (P = 0.01) \), BMI, WC and HC also decreased \( (P = 0.01, \ P = 0.001 \) and \( P = 0.01, \) respectively). The results (Table 6.3) show that the daily 5 g supplementation of \( C. \ cassia \) for eight weeks caused an average weight reduction almost 2 kg. As with the 4 week results, there were no significant changes in the anthropometric measurements observed in the placebo
In terms of blood pressure measurements, both the cinnamon and placebo groups had systolic and diastolic blood pressures were in the normal range for the baseline measurements. For the cinnamon treatment group, the systolic blood pressure was reduced at both weeks four and eight to 114.75 mm Hg and 105.42 mm Hg respectively ($P = 0.04$ and $P = 0.01$ respectively). The diastolic blood pressure decreased significantly at week eight to 73.28 mm Hg compared with the baseline 83.00±3.2 ($P = 0.02$). As with the other measurements taken, there was no change observed in the control group, suggesting the cinnamon supplement was having an effect on blood pressure, either directly or as a result of the changes to the other parameters measured.
Table 6.3 Changes in anthropometric measurements at week four and week eight compared with the baseline (values are mean values± SEM). * signifies a significant change (p values listed).

<table>
<thead>
<tr>
<th>Demographic</th>
<th>Cinnamon (n = 8)</th>
<th>Placebo (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Change at 4 weeks (Mean, SEM)</td>
<td>Paired t-test * p value</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>-1.00 ± 0.18</td>
<td>0.001*</td>
</tr>
<tr>
<td></td>
<td>-0.14 ± 0.26</td>
<td>0.60</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>-0.38 ± 0.06</td>
<td>0.001*</td>
</tr>
<tr>
<td></td>
<td>-0.05 ± 0.09</td>
<td>0.62</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>-1.56 ± 0.60</td>
<td>0.04*</td>
</tr>
<tr>
<td></td>
<td>1.21 ± 1.57</td>
<td>0.47</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>-0.65 ± 0.47</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>-0.35 ± 0.17</td>
<td>0.10</td>
</tr>
<tr>
<td>Systolic Blood Pressure (mm Hg)</td>
<td>-5.13 ± 2.14</td>
<td>0.04*</td>
</tr>
<tr>
<td></td>
<td>-4.00 ± 1.83</td>
<td>0.10</td>
</tr>
<tr>
<td>Diastolic Blood Pressure (mm Hg)</td>
<td>-3.25 ± 2.01</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>-1.86 ± 1.29</td>
<td>0.20</td>
</tr>
<tr>
<td>Resting Heart Rate (BPM)</td>
<td>-3.38 ± 1.67</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>-0.80 ± 4.10</td>
<td>0.85</td>
</tr>
</tbody>
</table>
6.4.4 Inflammatory marker levels at the baseline

6.4.4.1 Cytokines and C-reactive protein levels

The cytokine and CRP levels at baseline (Table 6.4) were within the normal range in both groups, and no significant differences between the two groups were evident. Furthermore, there was no significant difference in the inflammatory markers observed between the two groups, suggesting at baseline the groups are comparable and this is a good starting point for investigating any changes due to supplementation.

Table 6.4 Cytokines and CRP levels of study participants at the baseline.

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Cinnamon (n = 8) (Mean ±SEM)</th>
<th>Placebo (n = 7) (Mean ±SEM)</th>
<th>Unpaired t-test p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL2 (pg/ml)</td>
<td>Insufficient Data*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL4 (pg/ml)</td>
<td>Insufficient Data*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL6 (pg/ml)</td>
<td>1.11 ± 0.24</td>
<td>2.30 ± 0.82</td>
<td>0.17</td>
</tr>
<tr>
<td>IL8 (pg/ml)</td>
<td>4.35 ± 0.63</td>
<td>4.23 ± 0.73</td>
<td>0.90</td>
</tr>
<tr>
<td>IL10 (pg/ml)</td>
<td>0.88 ± 0.09</td>
<td>3.01 ± 1.57</td>
<td>0.17</td>
</tr>
<tr>
<td>VEGF (pg/ml)</td>
<td>Insufficient Data*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFN γ (pg/ml)</td>
<td>Insufficient Data*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNFα (pg/ml)</td>
<td>2.96 ± 0.53</td>
<td>5.56 ± 1.95</td>
<td>0.20</td>
</tr>
<tr>
<td>IL1β (pg/ml)</td>
<td>Insufficient Data*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL1α (pg/ml)</td>
<td>0.28 ± 0.02</td>
<td>0.96 ± 0.53</td>
<td>0.23</td>
</tr>
<tr>
<td>MCP1 (pg/ml)</td>
<td>97.70 ± 11.22</td>
<td>99.72 ± 16.10</td>
<td>0.92</td>
</tr>
<tr>
<td>EGF (pg/ml)</td>
<td>Insufficient Data*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>1.88 ± 0.26</td>
<td>1.56 ± 0.18</td>
<td>0.36</td>
</tr>
</tbody>
</table>

* The levels were below the level of detection of the high sensitivity cytokines kit used on the Evidence Investigator. Values are mean ± SEM, n=3.
6.4.5 Changes in cytokine levels after supplementation

6.4.5.1 IL-1β, IL-2 and IL-4

The pro-inflammatory cytokines IL-1β, IL-2 and anti-inflammatory IL-4 were measured at the baseline and after four and eight weeks of supplementation with either cinnamon or the control. Circulating levels were below the level of detection of the high sensitivity cytokines kit used on the Evidence Investigator therefore no further analysis was possible within this study. The limit of detection (LOD) of IL-1β, IL-2, and IL-4 were 0.26 pg/ml, 2.97 pg/ml and 2.12 pg/ml, respectively, which suggests the levels in the blood of the participants were very low.

6.4.5.2 VEGF and EGF

The concentration of VEGF and EGF were lower than the limit of detection of the high sensitivity cytokines kit used on the Evidence Investigator at all time points investigated, therefore the analysis of these growth factors was not possible. The LOD of VEGF and EGF were 3.24 pg/ml and 1.04 pg/ml, respectively, which again suggest the participants only expressed a low level in their blood.

6.4.5.3 IL-6

The levels of the pro and anti-inflammatory cytokine IL-6 were detectable in all samples at all-time points. The fasting serum IL-6 concentrations were within the normal range for both groups at baseline (Table 6.4), however the IL-6 concentrations were lower in the cinnamon group (1.11 pg/ml) compared with the control group (2.30 pg/ml, \( P > 0.05 \)), but the difference was not significant. A repeated measures two-way ANOVA (Figure 6.2) revealed that there was no significant effect of the cinnamon treatment on IL-6 levels (\( P = 0.300 \)). However, there was a significant change in IL-6 concentration due to time for treatment and control groups together (\( P = 0.010 \)). Interestingly, the level of IL-6 slightly increased at weeks four and eight for the cinnamon group, being 1.19 pg/ml and 1.59 pg/ml, respectively, however, using t-test this increase was not significant compared with baseline (\( P = 0.356, \ P = 0.245 \)). For the control group, the IL-6 concentration was similar for the baseline, and four and eight weeks. These results suggest the IL-6 levels increased over time for the cinnamon groups, but the differences were not significant when compared to the control group.
Figure 6.2: Fasting IL-6 concentration at baseline, week 4 and 8 for control and cinnamon groups. IL-6 levels were determined in each participant (n = 15) at week 0, 4 and 8. Individual values displayed with Mean ± SEM. ANOVA two-way analysis of variance, T treatment effect, D duration effect and D/T treatment and duration interaction effect.

6.4.5.4 IL-8

These results (Figure 6.3) show there was no effect of treatment on IL-8 levels, when compared to the control. The IL-8 concentration, however, changed significantly in the cinnamon group and the control group over time ($P < 0.001$), as cinnamon groups showed a significant decrease in concentration after four weeks of treatment when compared to the baseline, ($P = 0.33$, paired t-test) (Table 6.4), and then an increase again after eight weeks. However, there were no changes in control. This change was in the normal levels and the variability in cytokines levels during time is natural. Moreover, there was a significant interaction between treatment and time ($P = 0.003$, paired t-test), which suggests that the time had a greater effect on IL-8 concentration at cinnamon and placebo groups.
Figure 6.3: Fasting IL-8 concentration at baseline, week 4 and 8 for control and cinnamon groups. IL-8 levels were determined in each participant (n = 15) at week 0, 4 and 8. Individual values displayed with Mean ± SEM. ANOVA two-way analysis of variance, T treatment effect, D duration effect and D/T treatment and duration interaction effect.

6.4.5.5 IL-10

The serum anti-inflammatory cytokine IL-10 concentration in the cinnamon group was within the normal range (0.88 pg/ml) at the baseline (Table 6.4), whereas the level in the control group was higher than the Randox normal range, however, this level was normal according to the literature (Kleiner et al., 2013). The results (Figure 6.4) showed there was no significant effect of the treatment on fasting IL-10 concentration. However, the levels of IL-10 in the cinnamon group slightly increased from 0.88 pg/ml to 0.94 pg/ml and then to 1.07 pg/ml at weeks four and eight, respectively, whereas in the control group, IL-10 levels were very similar to the baseline at all-time points. Overall these results suggest there was a small but insignificant effect of the cinnamon on IL-10 values, which suggests it may have had an effect but this sample size study was not enough to observe an effect.
Figure 6.4: Fasting IL-10 concentration at baseline, week 4 and 8 for control and cinnamon groups. IL-10 levels were determined in each participant (n = 15) at week 0, 4 and 8. Individual values displayed with Mean ± SEM. ANOVA two-way analysis of variance, T treatment effect, D duration effect and D/T treatment and duration interaction effect.

6.4.5.6 TNF-α

At the baseline level (Table 6.4) both groups were within the normal range, with no significant differences between them, even though the control group had values two times higher than the cinnamon group. The results (Figure 6.5) showed that overall there was no significant effect of the treatment compared to the control (P = 0.644). The comparison of the duration for cinnamon and the placebo showed there was a significant effect on the TNF-α concentration in both groups (P = 0.002). In addition, there was a significant interaction between the effect of time and treatment (P = 0.008). The TNF-α concentration in the cinnamon group was decreased slightly at week 4 and 8 (2.82 pg/ml, 2.49 pg/ml, respectively) compared with baseline 2.96. However, the concentration of TNF-α in control was slightly increased compared with baseline. These results suggest the cinnamon supplementation did have a small effect on the TNF-α concentrations, as it caused a reduction, but the effect was too small to be significant in this study.
Figure 6.5: Fasting TNF-α concentration at baseline, week 4 and 8 for control and cinnamon groups. TNF-α levels were determined in each participant (n = 15) at week 0, 4 and 8. Individual values displayed with Mean ± SEM. ANOVA two-way analysis of variance, T treatment effect, D duration effect and D/T treatment and duration interaction effect.

6.4.5.7 IL-1α

The baseline IL-1α concentrations (Table 6.4) in the cinnamon group were within the normal range according to the Randox laboratory range and literature, however, in the control group the IL-1α levels (1.07 pg/ml) were high compared to the cinnamon group (0.28 pg/ml), however not significantly different (P = 0.23). These values were still within the normal range according to literature (Um et al., 2011). The results (Figure 6.6) suggest there was no significant effect of the cinnamon supplements on the IL-1α levels compared to the control. However, there was a significant change due to the effect of time on the IL-1α concentration in the control and cinnamon groups (P = 0.025), and a significant interaction between the effect of time and treatment (P = 0.044). There was a slight reduction in IL-1α levels in the cinnamon group at week eight (0.24 pg/ml) compared with the baseline (0.28 pg/ml), whereas the IL-1α levels in the control group increased gradually at weeks four and eight (1.16 pg/ml and 1.37 pg/ml respectively). Overall these results show there were minor changes in the levels
of IL-1α observed over time, but no specific difference was seen between the treatment and the control.

![Graph](image)

**Figure 6.6:** Fasting IL-1α concentration at baseline, week 4 and 8 for control and cinnamon groups. IL-1α levels were determined in each participant (n = 15) at week 0, 4 and 8. Individual values displayed with Mean ± SEM. ANOVA two-way analysis of variance, T treatment effect, D duration effect and D/T treatment and duration interaction effect.

### 6.4.5.8 MCP-1

The MCP-1 levels in both groups were within the normal range for the baseline (Table 6.4), with no significant differences between the groups. Even though the MCP-1 levels in the placebo group were slightly higher than in the cinnamon group (105.6 pg/ml and 98.1 pg/ml respectively). The results (Figure 6.7) suggest there was no effect of cinnamon supplements on MCP-1 concentration when compared to the control, whereas the time factor produced a significant change in MCP-1 concentration in both groups (P < 0.001), as the MCP-1 levels were reduced in the cinnamon and control groups at week four (86.66 pg/ml and 86.43pg/ml respectively), and then increased again slightly at week eight (96.44 pg/ml and 96.21 pg/ml, respectively). This suggests there was no specific effect of the cinnamon detected, but there was a variation in the
levels expressed over time within the study, in a similar pattern to that observed for IL-8.

![Graph showing MCP-1 concentration over time with annotations: T: P = 0.410, D: P = 0.000***, D/T: P = 0.383]

**Figure 6.7:** Fasting MCP-1 concentration at baseline, week 4 and 8 for control and cinnamon groups. MCP-1 levels were determined in each participant (n = 15) at week 0, 4 and 8. Individual values displayed with Mean ± SEM. ANOVA two-way analysis of variance, T treatment effect, D duration effect and D/T treatment and duration interaction effect.

### 6.4.5.7 hs-CRP

The hs-CRP concentration in the control and cinnamon groups at the baseline were within the normal range (Table 6.4), and the baseline hs-CRP concentration in the cinnamon group was 1.88 mg/l (Figure 6.8). Consuming cinnamon supplements for four weeks reduced significantly the hs-CRP concentration (1.26 mg/l, P= 0.016, paired t-test), however, there was no change in control (P = 0.800). The hs-CRP concentrations in the control group were almost the same at the baseline and after four and eight weeks (1.56 mg/l, 1.53 mg/l and 1.60 mg/l respectively). There were no significant differences over time or for the interaction between time and treatment, suggesting than cinnamon only had an effect on this marker. There was a positive correlation between hs-CRP and bone trabecular density mg per cm³ (BTrbDen...
mg/cm³) (R = 0.558, P = 0.031). Increasing bone trabecular density is associated with elevated hs-CRP concentration.

Figure 6.8: Fasting hsCRP concentration at baseline, week 4 and 8 of control and cinnamon groups. hsCRP levels were determined in each participant (n = 15) at week 0, 4 and 8. Individual values displayed with Mean ± SEM. ANOVA two-way analysis of variance, T treatment effect, D duration effect and D/T treatment and duration interaction effect.

6.4.6 Adhesion molecules levels at baseline

The blood concentrations of adhesion molecules can play a biological role in vascular inflammation and atherosclerosis. Therefore the adhesion molecules sVCAM, sICAM, sE-selectin (sESEL), sP-selectin (sPSEL) and sL-selectin (sLSEL) were selected for measurement.

There were no significant differences in the levels of the adhesion molecules at the baseline (Table 6.5) between the two groups, and the levels of sVCAM, sICAM, sESEL, sPSEL were within normal range. The sLSEL concentrations were slightly higher the normal range, and there was a positive correlation between age and sPSEL, suggesting the increased levels observed at the baseline were due to the age of the participants involved in the study.
Table 6.5 Adhesion molecule levels of the study participants at the baseline.

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Cinnamon (n = 8) (Mean ± SEM)</th>
<th>Placebo (n = 7) (Mean ± SEM)</th>
<th>Unpaired t-test p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>sVCAM1 (ng/ml)</td>
<td>626 ± 92</td>
<td>621 ± 45</td>
<td>0.965</td>
</tr>
<tr>
<td>sICAM1 (ng/ml)</td>
<td>305 ± 60</td>
<td>263 ± 16</td>
<td>0.535</td>
</tr>
<tr>
<td>sESEL (ng/ml)</td>
<td>20 ± 3.0</td>
<td>17 ± 1.50</td>
<td>0.329</td>
</tr>
<tr>
<td>sPSEL (ng/ml)</td>
<td>126 ± 11</td>
<td>117 ± 8.0</td>
<td>0.517</td>
</tr>
<tr>
<td>sLSEL (ng/ml)</td>
<td>2294 ± 407</td>
<td>1816 ± 158</td>
<td>0.319</td>
</tr>
</tbody>
</table>

Values are mean ± SEM, n = 3.

6.4.7 Changes in adhesion molecules levels after supplementation
6.4.7.1 sVCAM-1

The fasting soluble sVCAM-1 concentrations were measured at all three time points, and there was no significant difference between the normal levels of the groups at baseline (Table 6.5). In the cinnamon group (Figure 6.9) the levels of sVCAM-1 decreased from 626.0 ng/ml at baseline to 557.3 ng/ml and 494.3 ng/ml in weeks four and eight respectively, however, this reduction was not statistically significant. In the control group there was no change in the sVCAM-1 concentration at either time point compared with the baseline. The results (Figure 6.9) show there was no change in expression due to time, or interaction effect of time and treatment, which suggests the small change observed in the treatment was due to the cinnamon supplementation, but the difference was not significant.
Figure 6.9: Fasting sVCAM concentration at baseline, week 4 and 8 for control and cinnamon groups. sVCAM levels were determined in each participant \((n = 15)\) at week 0, 4 and 8. Individual values displayed with Mean ± SEM. ANOVA two-way analysis of variance, T treatment effect, D duration effect and D/T treatment and duration interaction effect.

6.4.7.2 sICAM-1

The sVCAM-1 concentration at the baseline (Table 6.5) in both groups was within the normal levels, and not significantly different. The fasting sICAM-1 levels for the control group (Figure 6.10) at both weeks four and eight were similar to the baseline and in the normal range. The sICAM-1 levels in the cinnamon group showed a reduction from 305.8 ng/ml to 276.1 ng/ml at week four and 232.3 ng/ml at week eight, though the difference was not at a significant level. The results showed there was no difference over time, or for the interaction between time and treatment.
Figure 6.10: Fasting sICAM concentration at baseline, week 4 and 8 for control and cinnamon groups. sICAM levels were determined in each participant (n = 15) at week 0, 4 and 8. Individual values displayed with Mean ± SEM. ANOVA two-way analysis of variance, T treatment effect, D duration effect and D/T treatment and duration interaction effect.

6.4.7.3 sESEL

There were no significant differences between the groups at the baseline (Table 6.5). In the cinnamon group (Figure 6.11) the sESEL concentrations at weeks four and eight decreased slightly in comparison with the baseline, at the baseline the sESEL levels were 20.1 ng/ml, after four weeks they were 18.9 ng/ml and after eight weeks they were 16.3 ng/ml, which is a measurable, but insignificant change. This difference may have been insignificant due to the spread of values in the cinnamon group, which would affect the error bars for each time point. In contrast, in the placebo group (Figure 6.11) the levels of sESEL at four (17.21 ng/ml) and eight weeks (16.49 ng/ml) were almost the same in the baseline (17.01 ng/ml), which suggest the change observed for the cinnamon group was due to the treatment. There was a positive correlation between sESEL and systolic blood pressure (R = 0.523, P = 0.045). Reducing blood pressure was associated with decreasing the concentration of sESEL. These suggest that reducing sESEL concentration play a role in reducing systolic blood pressure.
The results (Table 6.5) show that there were no significant differences between the groups at baseline, and there was no significant effect of taking cinnamon supplements on the sPSEL concentrations (Figure 6.12). However, there was a significant effect of time on the sPSEL values in the control and cinnamon groups ($P = 0.012$). The mean sPSEL concentration in the control group after four weeks was slightly increased (122.4 ng/ml) compared with the baseline (117.2 ng/ml), however, in the cinnamon group the fasting sPSEL levels marginally decreased at weeks four and eight ($P = 0.070$, $P = 0.261$). There was a positive correlation between sPSEL and systolic blood pressure, ($R = 0.512$, $P = 0.051$), and there was a positive correlation between sPSEL and age, ($R = 0.513$, $P = 0.050$). Elevated levels of sPSEL was associated with age and high blood pressure. Overall these results show that increasing levels of sPSEL was associated with age and systolic blood pressure.
Figure 6.12: Fasting sPSEL concentration at baseline, week 4 and 8 of control and cinnamon groups. sPSEL levels were determined in each participant (n = 15) at week 0, 4 and 8. Individual value displayed with Mean ± SEM. ANOVA two-way analysis of variance, T treatment effect, D duration effect and D/T treatment and duration interaction effect.

6.4.7.5 sLSEL

The baseline levels of sLSEL (Table 6.5) in the cinnamon group were moderately high (2122.5 ng/ml) according to the Randox laboratory range, however, it was normal compared to the literature (Schleiffenbaum et al., 1992; Zonneveld et al., 2014). After four and eight weeks of ingesting cinnamon supplements (Figure 6.13), the sLSEL concentrations slightly decreased to 1830.5 ng/ml and 1647.6 ng/ml, respectively, (P = 0.056, P = 0.288), however, this decrease did not reach statistical significance. sLSEL levels did not alter significantly within the control group, but there was a small increase after four (2029.52 ng/ml) and eight weeks (1881.17 ng/ml) compared to the baseline (1816.02 ng/ml). Overall these results show there was no significant effect of either treatment or time on sLSEL levels. As with the other markers investigated, these small changes may be due to the large variation within the samples affecting the size of the error bars and masking any actual change.
Figure 6.13: Fasting sLSEL concentration at baseline, week 4 and 8 for control and cinnamon groups. sLSEL levels were determined in each participant (n = 15) at week 0, 4 and 8. Individual value displayed with Mean ± SEM. ANOVA two-way analysis of variance treatment effect, D duration effect and D/T treatment and duration interaction effect.

Overall, these results show that a daily 5 g dose of cinnamon supplement for eight weeks caused a significant reduction in the BMI, and blood pressure, when compared to the placebo. This change could not be linked to a significant effect of treatment on the cytokines and adhesion molecules investigated, possibly due to the small sample size of the study. There was a significant effect of time on some of the cytokines and adhesion molecules investigated, including IL-6, IL-8, TNF-α, IL-1 α, MCP-1 and sPSEL, which suggests there were general changes in the levels of some the inflammatory markers that were investigated. Theses change in cytokines and adhesion molecules together indicate the wide variation in cytokine and adhesion molecule levels between individuals and within individual over time.
Chapter 6

6.5 Discussion

The inflammatory response needs to be tightly regulated as the pro-inflammatory cytokines, such as TNF-α, IL-1 and IL-6, produced during the typical inflammation response can cause damage to normal tissues. For example, a delay in this regulated process, and the associated scavenging reactions, may lead the inflammatory response observed in a variety of chronic inflammatory diseases, such as atherosclerosis and arthritis (Hong et al., 2012). Some NSAIDs are commonly used to treat inflammation, however, they have some undesirable side effects, such as increased blood pressure and stomach ulcers (Gunawarden et al., 2014). The side effects of these drugs provides the motivation for the development of natural anti-inflammatory treatments, with potentially fewer side effects, for example some preliminary studies have reported that cinnamon has anti-inflammatory properties (Kwon et al., 2011). Cinnamon is a traditional medicine that has been used for thousands of years in ancient Egypt and China (Lu et al., 2011). In vitro and in vivo studies have shown that cinnamon contains biologically active substances that can mimic insulin’s properties and can lower the blood sugar in uncontrolled type 2 diabetics (Khan et al., 2003; Baker et al., 2008). Moreover, many studies have investigated the anti-microbial and anti-cancer properties of cinnamon, both in cells and in animals (Qin et al., 2010; Ranasinghe et al., 2013). Currently the research into the anti-inflammatory properties of cinnamon is limited to that in animals and cells, but with positive results, therefore, it is worth determining the health effects of cinnamon as an anti-inflammatory in humans. As far as we know, this study is the first research investigation into the effect of *C. cassia* supplements on extensive profiles of cytokines and adhesion molecules in overweight healthy subjects.

There is evidence that cinnamonaldehydes isolated from *C. cassia* inhibit COX-2 and NOS in vitro, which may be responsible for the anti-inflammatory and anti-oxidant properties observed for cinnamon (Lee et al., 2005; Kim et al., 2007). Furthermore, administering a *C. cassia* water extract (42.6mg/ml) to mice, which were injected with lipopolysaccharide (LPS) (inducer of TNF-α), for 6 days caused a significant reduction in serum levels of TNF-α and IL-6, suggesting it had anti-inflammatory properties (Hong et al., 2012).
The current data with regards to the cytokine profiles of healthy overweight and older individuals is limited, but there is evidence that the circulating levels of pro-inflammatory cytokines such as TNF-α and IL-6 increase with age, and other factors (Cauley et al., 2007; Prasad et al., 2012). Furthermore, elevated CRP levels have been found to be associated with developing type 2 diabetes three to four years earlier in elderly individuals (Barzilay et al., 2001). Although previous studies have found a positive correlation between an increase in cytokine levels and ageing (Franceschi et al., 2007; Park et al., 2011), which would mean that older people should have high levels of cytokines. Our cytokine and CRP results did not show this increase or correlation with age. However, the results for the levels of the adhesion molecule PSLE was positively correlated with age ($R = 0.513$, $P = 0.050$). This correlation might be influenced by environmental factors such lifestyle and smoking, however the participants involved in this study were non-smokers (Woodward et al., 2003; Ferrucci et al., 2005). Overall the results suggested the levels of cytokines in the participants were within the normal range. Furthermore, Singh and Newmana (2011) reported that inflammatory cytokine levels increased during the menopause. Although, the participants were in the menopause stage, their cytokine levels were within the normal range, suggesting the menopause and age did not have a significant effect in this group, possibly due to the small sample size.

Our results showed that some of the inflammatory cytokines investigated reduced slightly after consuming cinnamon supplements, whilst others increased. One of these markers was IL-6, whose concentration increased significantly in the cinnamon and placebo groups due to the effect of time ($P = 0.01$). The IL-6 levels in the cinnamon group increased slightly at weeks four and eight compared with baseline. However, this was different in the control group, where the level of IL-6 did not change over time. IL-6 is produced by a variety of cells such as vascular endothelial cells, immune cells and adipocytes, and has been shown to have anti-inflammatory and pro-inflammatory properties (Singh and Newman, 2011). The ability to perform both anti-inflammatory and pro-inflammatory functions is based on its interaction with the IL-6 receptor; when IL-6 binds to the soluble interleukin-6 receptor (sIL-6R), which is called trans-signalling, it activates the pro-inflammatory pathway. However, when IL-6 binds with the membrane interleukin-6 receptor (mbIL-6R), which is called classic signalling, it activates the anti-inflammatory pathway (Scheller et al., 2011). Thus, it may be that
cinnamon activates the anti-inflammatory pathway of IL-6, which would explain why our results showed the IL-6 concentrations tended to increase at weeks four and eight which corresponded to a reduction in BMI. Studies have shown that CRP levels can relate to directly to the IL-6 concentration (Ganesan et al., 2005). The CRP data in this study showed no significant change in CRP levels, however, there was a slight decrease in the cinnamon group, which could be linked to the changes observed in IL-6 levels.

Obesity is also associated with high levels of IL-6, as well as an IL-6 deficiency (Wallenius et al., 2002; Pini et al., 2013). Studies have shown that injections of a low dose of IL-6 into IL-6 deficient obese mice can lead to a reduction in their weight (Wallenius et al., 2002). Therefore it can be speculated that keeping IL-6 levels at the high end of the normal range might help in reducing weight. The participants in this study who consumed the cinnamon supplement lost almost 2 kg during the 8 week duration. This suggest that the slight increase in IL-6 concentrations may therefore play a role in helping the cinnamon group individuals to reduce their weight.

There is some evidence that elevated levels of inflammatory markers such as IL-6 have a negative effect on carbohydrate metabolism (Lazar, 2005). Whereas other studies show that acute IL-6 treatment increased insulin-stimulated glucose disposal (uptake) in humans, and increase fatty acid oxidation in vitro (Carey et al., 2006). These studies suggest that IL-6 is involved in a range of biological activities in the body that are not solely concerned with inflammation. However, using cytokines for human treatment must be undertaken with caution due to their potential side effects (Carey et al., 2006). The use of other methods, such as diet and herb supplements, might be a safer alternative way to have an effect on cytokines. Although the significant increase in IL-6 concentrations in this study were due to the effect of time, the mean IL-6 concentrations in the cinnamon group at weeks four and eight increased, whereas the means in the control group did not change. However, this increase in the cinnamon group was within the normal range according to the literature (Kim et al., 2011; Martins et al., 2011) and the Randox laboratory.

Another reason for the different concentrations of IL-6 measured at 4 and 8 weeks in our study, might be due to the time of the year. This study started in the spring and was completed during the summer, which can have an effect on IL-6 levels. To make
sure this increase did not have a negative effect on the cinnamon participants, the fasting levels of hs-CRP were determined at the baseline and after four and eight weeks, for both the cinnamon and control groups. Hs-CRP is an acute phase protein produced by the liver in response to an elevation in IL-6, and is regulated in the liver by IL-6, IL-1 and TNF-α (Terra et al., 2009). Several studies have shown that IL-6, TNF-α, and hs-CRP increased with age in healthy and in overweight or obese individuals (Singh and Newman, 2011), but this was not noticeable in our study for the participants at the baseline, and during the treatment, although they were older (over 53 average age), and overweight but healthy. The Pearson correlation that was used in our data showed that there was no correlation between the age and cytokines and CRP levels, and there was no significant effect of cinnamon supplements on TNF-α and hs-CRP, which suggests the increased IL-6 levels did not have a negative effect on the participants. In a study by Biancotto et al. (2013) 27 cytokines levels were determined in 144 healthy participants, aged between 21-62 year, at baseline and after 7 days. Their outcome showed a higher viability between the cytokine levels over time. Thus, the variability is expected in the cytokine levels over time in healthy people.

Some studies have shown that certain inflammatory markers are associated with bone loss or osteoporosis, for example IL-6 and hs-CRP have a negative relationship with bone mineral density (Gough et al., 1998; Oelzner et al., 1999). However, a study by Ganesan et al. (2005) found that the plasma hs-CRP concentration does not correlate with bone mineral density, and Kania et al. (1995) highlighted that plasma IL-6 did not correlate with bone mineral density either. Our results have shown that the serum levels of hs-CRP had a positive correlation with bone trabecular density (BTrbDen mg/cm3) ($R = 0.558, P = 0.031$) that mean decreasing BTrbDen was associated with reducing CRP levels. However, Rolland et al. (2012) stated that hsCRP levels over 5 mg/L were associated with lower BTrbDen in men after the age of 72 years. This correlation demonstrated by Rolland et al. (2012) were more obvious in the participants who had CRP concentrations above the normal range, which is >5 mg/L, and aged over 72 years. In addition, the participants in their study were men who may respond differently to women.

In addition, the menopause is correlated with elevated levels of hs-CRP, which has also been shown to be significantly associated with CVD in women (Woodward et al., 2003), and also elevated levels of hs-CRP have been identified as a risk factor in the
development of diabetes in women (Freeman et al., 2002). This study has shown that the participants' hs-CRP concentrations were not high and within the normal range. These normal levels were possibly due to the participants having passed the menopausal period, as the mean age was 53 years in the control group and 56 years in the cinnamon group. The mean BTrbDen mg/cm³ values in cinnamon and control were 182.70 ± 14.39, 167.42 ± 11.74, respectively. Both groups were in the menopausal age range and the density values would tend to be reduced in postmenopausal women (Grampp et al., 1997). These values were similar to other data on BTrbDen mg/cm³ in postmenopausal women (175 ± 53) (Grampp et al., 1997).

The outcome of this study illustrated there was a significant reduction in weight for the participants that consumed 5 g of cinnamon for 8 weeks. There is evidence of a connection between obesity and systemic inflammation, for instance, some human studies have shown increased TNF-α expression in adipose tissues in obese individuals with a reduction in TNF-α after losing weight (Terra et al., 2009). Studies have shown that adipose tissues of obese individuals express increased amounts of pro-inflammatory cytokines, such as IL-6, IL-1, TNF-α, IL-8, CRP, sICAM and MCP-1, and that the adipose tissue may activate the inflammatory cascades that are associated with a chronic low grade systemic inflammation, which can develop in many diseases, such as type 2 diabetes (Terra et al., 2009; Leherer et al., 2013). In addition, adipose tissues may play a role in the pathogenesis of hypertension via releasing inflammatory cytokines (Ghanem et al., 2007). These studies suggest that there is association between cytokine levels and weight reduction. Our study suggests that 5 g of cinnamon has a slight effect on cytokine concentrations in the cinnamon group, such as IL-6, TNF-α and CRP, whereas, there was no change within the control group. The anti-inflammatory IL-10 concentration increased slightly during the treatment from 0.88 pg/ml at the baseline to 1.07 pg/ml at week eight in the cinnamon group, but this was not a significant change. IL-10 is a cytokine which suppresses the immune response and inhibits the production of pro-inflammatory and systemic inflammatory factors such as TNF-α and CRP (Lee et al., 2002). This cytokine is important as a balance between the pro-inflammatory and anti-inflammatory factors and leads to the recovery of homeostasis, which is very important in the effective inflammatory response against pathogens (Marie et al., 1996; Franceschi et al., 2007).
Moreover, it is possible that cinnamon contains biological substances that helped the cinnamon participants to significantly reduce their weight and keep the balance between pro-inflammatory and anti-inflammatory factors. Anderson et al. (2004) reported that the active compounds in cinnamon were A-type proanthocyanidins, which give cinnamon its antioxidant properties and other health benefits, such as controlling glucose levels in humans. Proanthocyanidins are a type of polyphenol (condensed tannins) that are oligomers and polymers made up of monomeric flavan-3-ols, such as catechin and epi-catechin (Passos et al., 2007). A-type proanthocyanidins prevent bacterial adhesion in bacterial urine infections in vitro, but B-type proanthocyanidins do not have this propriety (Howell et al., 2005; Prior et al., 2010). Various studies conducted in vivo and using in vitro models have provided evidence that natural polyphenols from plant extracts can modulate intestinal inflammation (Andújar et al., 2012).

Recent studies have shown that procyanidins have anti-inflammatory properties in rats and mice (Terra et al., 2009). The rats were fed procyanidins from grape seeds (345 mg/kg) for 19 weeks, which significantly reduced the plasma CRP and TNF-α (P < 0.05). Furthermore, feeding a cinnamon water extract orally at doses of 20 and 100 mg/kg to mice for 6 days, resulted in a significant reduction in IL-6 and TNF-α levels (Hong et al., 2012), showing both treatments produced anti-inflammatory effects. In a recent study, Gunawardena et al. (2014) found that cinnamon extract inhibited TNF-α production in RAW 264.7 macrophages. They highlighted that the most active phytochemical compounds in cinnamon that provided the anti-inflammatory properties were E-cinnamaldehyde and o-methoxycinnamaldehyde. Further evidence of the anti-inflammatory effects comes from a study that showed an aqueous C. cassia extract (10 µg/ml) and C. cassia inhibited 82 % of the activity of COX-2 in mouse macrophages (RAW 264.7 cells line) (Hong et al., 2002). All these study results show that cinnamon is a potential medicinal herb that has a capacity to inhibit the excessive modulation of the inflammatory process, such as cyclooxygenases-2 (COX-2) and cytokines, which means cinnamon could be used as alternative anti-inflammatory agent. The anti-inflammatory properties of cinnamon were not detected in this study possibly due to the small sample size, which limited the power of the study.
6.5.1 Adhesion molecules and blood pressure

The risk of CVD has been found to be associated with an elevation in some of the adhesion molecule levels in circulation, therefore evaluating the biomarker of inflammation in circulation, including adhesion molecules, might be a useful tool for identifying a patient at high risk of future CVD (Pak et al., 2014). Hypertension is defined as a systolic blood pressure greater than 140 mm Hg or diastolic greater than 90 mm Hg (Medina-Remón et al., 2013). High blood pressure is one of the major risk factors for developing CVDs such as coronary disease, stroke and atherosclerosis (Kannel, 2000). In the review by Ghanem et al. (2007) they highlighted a potential link between inflammation and hypertension. The results of this study confirmed there was a positive correlation between sPSEL and systolic blood pressure ($R = 0.512, P = 0.051$), and in addition there was a positive correlation between sESEL and systolic blood pressure ($R = 0.523, P = 0.045$), which suggest our results agree with Ghanem et al. (2007), that sPSEL and sESEL were positive correlated with hypertension.

Within our study there were no significant differences between the sVCAM, sICAM, sESEL, sPSEL levels at the baseline for the cinnamon and control groups, whereas the sLSEL concentrations at the baseline were slightly higher than the normal range that are based in the Randox protocol, but they were at a level that was considered within the normal range according to the literature (Schleiffenbaum et al., 1992; Zonneveld et al., 2014). Studies have shown that consuming alcohol alters adhesion molecule metabolism, which leads to increased serum levels of adhesion molecules such as sICAM, sESEL and sLSEL (Ponthieux et al., 2004). For this reason, in the study protocol, the participants were asked to avoid consuming alcohol for 3 days prior to the study day. However, it seems that some of the participants did not follow this instruction, which resulted in the baseline readings having a slightly higher levels of sLSEL. Moreover, adhesion molecule levels, such as sVCAM, sICAM, and sL-selectin increase with age (Nash et al., 1996). Hence, elevated levels of circulating adhesion molecules were expected within this age group, and was confirmed for sPSEL as it had a positive correlation with age ($R = 0.513, P = 0.050$). Adhesion molecules such as sVCAM, sICAM, sESEL, sPSEL and sLSEL have also been shown to play a role in endothelial function; they are involved in the recruitment leukocytes to sites of effected tissue (Etzioni., 1996), and they are present on the cell surface of vascular endothelial cells. There is evidence that inflammatory cytokines regulate the adhesion and
migration of leukocytes across the endothelium, which suggests their levels should correlate (Carlos and Harlan, 1994).

Lifestyle approaches are a widely used effective way to lower blood pressure or cardiovascular risk associated with hypertension, this can include smoking cessation, physical exercise, weight reduction and increasing plant, fruit and vegetable intake (Mancia et al., 2007). In recent years the focus on the management of blood pressure has shifted away from pharmacological conventional approaches, towards plant therapeutic powers, with or without standard medication (Tabassum and Ahmad, 2011). Plants, fruit and herbs may contain a specific component that reduces blood pressure, and these can work in a variety of ways, for example herbs, seeds, tea and vegetables are rich in polyphenol compounds, which provide the antioxidant capacity of these foods (Perez-Vizcaino et al., 2009). Flavonoids are one group belonging to the polyphenol family, they are important plant metabolites and can be used to control high blood pressure (Perez-Vizcaino et al., 2009). For instance, grapes and grape seeds contain a high concentration of antioxidant polyphenols such as flavonoids, anthocyanins and procyanidins (Packer et al., 1999; Zern and Fernandez., 2005). In a study by Barona et al. (2012), the participants with MetS and high risk of developing high blood pressure consumed 46 g/d of grape polyphenol supplements for 30 days in a double blind crossover design. The results of this study showed that participants who used grape capsules had a reduction in the risk factors associated with the metabolic conditions. Participants who received the grape polyphenol for 30 days had a significant decline in systolic blood pressure to 122 mm Hg, while those who received the placebo had a systolic blood pressure of 128 mm Hg. In addition, there was a reduction in sICAM-1 concentrations by 10 µg/L, (142 ± 50 µg/L) in grape group compared with placebo (151 ± 51 µg/L), whereas the plasma sVCAM-1 levels did not differ between the grape (1020 ± 285 µg/L) and placebo groups (1020 ± 240 µg/L). Overall the study showed that the changes in sVCAM-1 concentrations were positively correlated with changes in systolic blood pressure. Further evidence for the effect of polyphenols comes from a review of studies that showed consuming cocoa and dark chocolate lowered blood pressure in human beings in short-term studies. Cocoa contains high levels of proanthocyanidins (procyanidins), which comprise 58 % of the total polyphenol composition, which suggests these are the molecules that are having the effect on blood pressure (Andújar et al., 2012; Medina-Remón et al., 2013). Some
preliminary studies have reported that cinnamon reduces blood pressure in humans, though according to recent meta-analysis, there were only three randomised controlled trials that study the effect of cinnamon on blood pressure (Akilen et al., 2013). In our randomised controlled trial, 5 g per day of *C. cassia* supplements given for eight weeks reduced systolic and diastolic blood pressure significantly, ($P = 0.01$, $P = 0.02$, respectively). This suggests that cinnamon, grape and cocoa contain the same active compounds which reduce blood pressure, such as the polyphenol and in particular procyanidins. Our LC-MS data (chapter three) agrees with this theory as it confirmed that *C. cassia* contains procyanidins.

Within our study there was no significant difference between the groups at the baseline for diastolic blood pressure, however, the systolic blood pressure between the cinnamon (119 ± 8 mm Hg) and control groups (108 ± 9.4 mm Hg) was significantly different ($P = 0.03$). The difference between the groups in human experiments can occur by chance, possibly due to the lifestyle characteristics of the participants. Another reason for the difference could be due to the control group’s age as they were somewhat younger than the cinnamon group, and as age increases as blood pressure increased. After four weeks of treatment, the systolic and diastolic blood pressures were significantly reduced (-5.16 mm Hg and -3.25 mm Hg, respectively) in the cinnamon group, whereas there were no reductions in the placebo group. Also after 8 weeks, cinnamon treatment decreased the systolic and diastolic blood pressures significantly (-14 mm Hg and -9.14 mm Hg, respectively), compared to the baseline, with no difference in the control. This positive effect of cinnamon on blood pressure has been confirmed by two other studies; in the Akilen et al. (2010) study, the cinnamon (*C. cassia*) dose was 2 g/d for 12 weeks (N = 58), which significantly reduced the systolic and diastolic blood pressure ($P < 0.001$) of the cinnamon group. The baseline of the systolic and diastolic blood pressure on the cinnamon group was 133 mm Hg and 85 mm Hg respectively, which dropped to 129 mm Hg and 81 mm Hg, respectively, after treatment. For the placebo group the baseline of the systolic and diastolic was 135 mm Hg and 86 mm Hg, which changed to 134 mm Hg and 87 mm Hg, respectively after treatment. Another study found that ingesting 500 mg of cinnamon extract (Cinnulin PF®) daily, which is equivalent to 10 g of cinnamon, for 12 weeks reduced systolic blood pressure within the cinnamon group (n = 12) compared with the placebo group (n = 10) (Ziegenfuss et al., 2006). On the other hand, Wainstein et al. (2011)
only found a marginal effect ($P = 0.06$) on blood pressure of 1.2 g/d of cinnamon for 12 weeks ($n = 59$). This result might be because they only used a low dose of cinnamon, and it is likely that the effect of cinnamon on blood pressure is dose dependent. Also, in the previous study, the baseline of blood pressure was high, and the higher the blood pressure the greater chance it has to decrease, which meant the participants had a reduction in their blood pressure. However, in our study the participant’s blood pressure was within the normal range, and therefore our results show that 5 g per day of cinnamon supplements has a potential benefit for hypertensive individuals. Also, it could be considered as an additional dietary supplement option to regulate blood pressure levels along with conventional medication, or to prevent high blood pressure.

There are many possible explanations for the significant reduction in blood pressure after being given cinnamon supplements; there is growing evidence from controlled trials that dietary flavonoids and polyphenols can improve the endothelial function and reduce blood pressure (Hodgson et al., 2006; Barona et al., 2012). Polyphenol antioxidants are used to protect endothelial cells against reactive oxygen species (ROS), and they can induce the formation of nitric oxide that is responsible for relaxation of the blood vessels, which permit easy flow of blood through the vessels, thus lowering the blood pressure (Andújar et al., 2012). Damaged endothelial cells, due to exposure of oxidative stress, reduces the production of nitric oxide, and as a result, endothelial cells of the blood vessels expressed high concentrations of adhesion molecules, which might lead to increase blood pressure (Barona et al., 2012; Medina-Remón et al., 2013). Studies have shown that high levels of ROS increased the expression of adhesion molecule such as VCAM (Cook-Mills et al., 2011), therefore reducing adhesion molecule levels could increase the antioxidant status. In this study the sVCAM, sICAM, sE-selectin, sP-selectin and sL-selectin concentrations were slightly (but not significantly) reduced in cinnamon group only. Overall these results suggest that the cinnamon contains a biological active compound that helped to significantly reduce the blood pressure and decreased the adhesion molecule markers. It is possible that cinnamon procyanidins improved the endothelial function and slighter reduction in adhesion molecules concentrations. Consequently, this might lead to a significant reduction in blood pressure within the cinnamon group after ingesting 5g of $C. cassia$ for eight weeks. The study did not see a significant treatment effect in sVCAM, sICAM, sESEL, sPSEL and sLSEL possibly because the number of
participants was small, which reduced the study power. The effect at 90% power required 18 individuals for the cinnamon group and 18 for the placebo group. Even though many methods were employed to recruitment participants, the total number that were enrolled, and met the criterion of the study, was only 15 healthy women. Moreover, the time of the year, which was during the summer holidays, may have had an effect on recruitment, along with the age criterion which was limited to the middle-aged (45-70 years), healthy women.

This study also found no correlation between adhesion molecules markers, which include sVCAM, sICAM, sESEL, sPSEL and sLSEL, and BMI. This result was consistent with the work by Turan et al. (2014) who found there was no correlation between ICAM, VCAM and E-selectin levels and BMI.

Overall, this study showed 5 g of cinnamon supplements taken daily for eight weeks significantly lowered blood pressure and significantly reduced the weight. Cinnamon had no effect on the concentrations of cytokines and adhesion molecules investigated, possibly due to the small sample size. However, some adhesion molecules were slightly (but not significantly) reduced sVCAM, sICAM, sESEL, sPSEL and sLSEL, which may have led to an improved endothelial function, and that might be responsible for the reduction in blood pressure.

### 6.6 Conclusions

The present study showed that ingesting 5 g of *C. cassia* supplements every day for eight weeks displayed important health benefits in healthy overweight woman. During the intervention with supplements, the cinnamon participants’ weight was reduced significantly, by approximately 2 kg during the eight weeks. Moreover, blood pressure was reduced significantly at both weeks four and eight. Cinnamon supplements did not have a significant effect on the inflammatory marks such as IL-6, TNF-α, IL-10 and hs-CRP. Similarly, ingesting 5 g of cinnamon for eight weeks did not change the adhesion molecules such as sVCAM, sICAM and sLSEL, significantly. Interestingly, there was a slight reduction in some of the markers in the cinnamon group, such as sVCAM, sICAM, sESEL, sPSEL, sLSEL, TNFα and hs-CRP concentrations. However, the concentrations of most inflammatory markers within the control were not changed, possibly due to the small sample size. As a result of the significant effect of *C. cassia*
on weight and blood pressure, further research is required to assessed the effect of *C. cassia* in lipid profiles and glucose levels in humans.
CHAPTER SEVEN
Chapter 7

The effect of consuming a *Cinnamomum cassia* supplement on lipid profiles, glucose and insulin levels in healthy overweight participants over eight weeks

7.1 Introduction

Metabolic syndrome (MetS) is a collection of conditions that includes: high blood pressure, insulin resistance, an excess of body fat around the waist, high cholesterol and high fasting glucose concentrations (Dai et al., 2015). MetS increases the risk of developing CVD and type 2 diabetes, and as such is an important syndrome to treat (Grundy., 2008). Studies have shown that increased non-esterified fatty acid (NEFA) levels were associated with insulin resistance and high blood pressure (Maison et al., 2000). Therapeutic agents such as acarbose, and metformin, are used to manage hyperglycaemia; an Angiotensin converting enzyme (ACE) inhibitor such as captopril can be used to treat high blood pressure, and statins can be used to lower cholesterol in the blood. However, all of these therapies are associated with side effects, such as abdominal discomfort, diarrhoea and flatulence, therefore being able to use a treatment without side effects would be preferable (Phillips et al., 2003; Sönmez et al., 2005).

Complementary and alternative medicines, such as herbal medicines, have been used as an alternative approach to meet the health needs of patients providing safe, effective and low cost remedies for a variety of disorders and diseases (Barnes et al., 2008). Although plant and herbal products have been used in traditional medicine for centuries, the effective attributes of these have only recently been confirmed experimentally (Kennedy, 2005; Opara and Chohan, 2014). The use of herbal and natural supplements are widespread in the adult population of the USA, which has increased from 50.6 million in 2002 to 55.1 million in 2007 (Wu et al., 2007). Of these in 2002, there were 38.2 million adults in the United States who used herbal supplements to treat a specific condition such as a chest cold, stomach illness, diabetes and hypertension (Kennedy, 2005). The National Health Interview Survey (NHIS), conducted in the United States in 2007, showed that approximately 18 % of adults and 4 % of children used herbal supplements as an alternative medicine, and
this was found to be the most common complementary therapy. Moreover, $14.8 billion was spent on herbal products in the USA in 2007 (Camiel et al., 2013). More strikingly, about 50% of the population in Australia use herbal medicines on a regular basis (Wohlmuth et al., 2002). Furthermore, in Saudi Arabia, about 58.89% of 518 participants in survey 2010 used herb medicine (Elolemy et al., 2012). A recent study in Saudi Arabia showed that 42.3% of 292 participants questioned used herbs to treat disease (Mohammad et al., 2015). Survey data in 1998 in the UK showed that 28.3% of British population used herbal medicines (Thomas et al., 2001). All of the above statistics show that people choose to take herbal medicines as alternatives to conventional medicines, due to the supposed low levels of toxicity and their natural origins. Although herbal medicines may have several physiological beneficial actions on the human body, their underlying mechanisms are largely unclear (Opara and Chohan, 2014). There is therefore a need for more research to assess the actions of herbal medicines in order to benefit both the current users of herbal products, and to contribute to the development of new pharmaceutical products for the prevention and management of human diseases such as type 2 diabetes (Fabricant and Farnsworth, 2001). For instance, metformin, which is a drug that lowers blood glucose by increasing the sensitivity of the body’s cells to insulin in type 2 diabetes, is derived from French lilac, which is a herbal remedy that was used to treat diabetes in the Middle Ages (Ryan et al., 2001). As such further effective treatments may be derived from other plant and herb sources.

Studies have shown that the incidence of type 2 diabetes is increasing rapidly, and this to a great extent is due to the obesity epidemic (Lazar 2005). Changes in lifestyle, such as an improved diet and an increase in physical activity, can help to control blood glucose levels and go some way to preventing the development of type 2 diabetes (Buttar et al., 2005; Lichtenstein et al., 2006). Herbs and spices, such as cinnamon, cloves, tea, oregano and nutmeg, have been shown to have an insulin-like activity in vitro (Broadhurst et al., 2000). Therefore the role of herbs and spices in the treatment of diabetes and other diseases is of great interest.

Cinnamomum cassia (C. cassia) belongs to the Lauraceae family, and has traditionally been used in the treatment of disease. C. cassia has been approved as a medicinal herb by the German Commission E and the European Scientific Cooperative on Phytotherapy (ESCOP) (Blumenthal et al., 1998). The main components in C. cassia
bark are the non-volatile compounds (mainly condensed tannins), comprising 23.2 % proanthocyanidins, 3.6 % epi-catechins (Shan et al., 2007). Some preliminary studies suggest that C. cassia has beneficial effects on glucose uptake, and it contains polyphenols, which can stimulate glucose uptake and glycogen synthesis in vitro (Solomon and Blannin, 2007). It has also been reported that cinnamon has strong antioxidant, antibacterial, anti-inflammatory, and anti-cancer properties, and can be used in the treatment of common colds, CVD, and chronic gastrointestinal complaints (Verspohl et al., 2005; Kim et al., 2006; Molania et al., 2012; Hong et al., 2012).

This chapter focuses on the effects of C. cassia supplementation on a variety of parameters related to glucose and lipid metabolism, as well as body weight and food intake in overweight women over an 8 week period.

### 7.2 Study aims

1. To investigate the effect of C. cassia supplementation (5 g) on fasting blood glucose levels in overweight women between the ages of 45-70 years.

2. To investigate the effect of C. cassia supplementation (5 g) on lipid profiles (CHO, HDL, LDL, TAG, NEFA) in overweight women between the ages of 45-70 years.

3. To investigate the effect of C. cassia supplementation (5 g) on homeostasis model assessment (HOMA) in overweight women between the ages of 45-70 years.

4. To investigate the effect of C. cassia supplementation (5 g) on weight and anthropometric measurements in overweight women between the ages of 45-70 years.

5. To compare the effects of C. cassia supplementation (5 g) on food intake.

### 7.3 Methods

#### 7.3.1 Subjects

This study took the form of a randomised controlled trial (RCT). Approval for the study was granted by the University of Surrey’s Ethics Committee (EC/2013/70/FHMS), (Appendix 8). A power calculation for the study was carried out to estimate the numbers required; this was based on previous studies which examined fasting glucose levels, as this was considered the primary outcome variable of this study.
A reduction in fasting glucose of 2 mmol/l was observed by the cinnamon supplementation study of Khan et al. (2003), therefore the standard deviation was assumed to be 1.0. The power of the study was calculated at 90%, so 18 participants were required for each group; one group would be required to take cinnamon supplements and the other group the placebo. In order to allow for participants dropping out, a 25 percent margin was added to this; thus 23 participants were required for each group.

The researcher produced a poster (Appendix 9) about the study and placed copies around the University site, as well as e-mailing University staff. The locations of the posters included train stations and bus stops, restaurants and hotels, supermarkets and churches, as well as the hospitals in Kingston, Woking, Guildford and other locations. The researcher also presented a talk at Woking Mosque about the study. Despite all of these efforts, only 16 women in total volunteered and were found to meet the study’s criteria.

### 7.3.2 Screening

A screening process was carried out to ensure the eligibility of the participants. The screening was also used to make sure that the subjects were healthy enough to be included in the study. Participants were asked to fast overnight before screening for blood glucose levels. This was done in the morning using a finger prick test at the Clinical Investigation Unit (CIU) at the University of Surrey. The participants’ weight and height were also measured and they were asked to complete a screening questionnaire (Appendix 10). This featured questions concerned their health and lifestyle. The participants were given a participant information sheet (Appendix 11) and a consent form (Appendix 12).

### 7.3.3 Study participants

In order to assess whether or not the participants were suitable for the study, the following inclusion criteria were applied:

1. Female and in the age range of 45 to 70 years old
2. Not on regular medication
3. Not allergic to cinnamon
4. Body Mass Index (BMI) of 25 or more
5 Non-smoker
6 Generally in good health; not suffering from any diseases
7 Not taking any herbal supplements or other supplements in the last month
8 Willing and able to comply with the procedures required by the study

7.3.4 Study design

The study (Figure 7.1) followed the design of a randomised controlled trial (RCT) and included a control group using random number table. A cinnamon (C. cassia) supplement which was supplied by NBTY Inc, and purchased from Puritan’s Pride, was used in this study. Participants took the supplements for eight weeks, and during that time they visited the CIU three times. New dark green capsules (CapsulCN®) were filled (2.2.11.3) for cinnamon group with 5 g of cinnamon (C. cassia). The same capsules were filled with 5 g of corn flour for the control group. Each participant took three capsules after breakfast and three after their evening meal for the eight weeks of the trial. As the capsules looked the same, the participants were unaware of whether they were consuming cinnamon or the placebo.

In preparation for each study day, all participants consumed a standardised meal 400 g of vegetable lasagne with a known macronutrient composition the evening before. They were then asked to fast overnight for at least 12 hours. The standardised meal was used to reduce the variability between the volunteers. The participants arrived at the CIU early in the morning, having not consumed any alcohol or participated in any strenuous exercise for the previous three days. They were also asked not to drink any liquids which contained caffeine (coffee/tea/soft drinks such as Coca Cola) 24 hours prior to each visit. For three days prior to attending the CIU, the participants also kept a food diary and recorded their food consumption. The macronutrient content of the foods consumed were then calculated using DietPlan version 6 software. Blood pressure, fasting blood samples and anthropometric measurements were taken at each week.

At the baseline study day only, Peripheral Quantitative Computed Tomography (pQCT) was used in scanning mode of the participants’ wrist bones. Additionally, on the first and third visits, the participants were given a drink containing 75 g of glucose, and blood samples were taken again after two hours. This was a standard oral glucose tolerance test (OGTT). When all of the tests had been completed and all of the
necessary information had been collected, the participants were offered breakfast, and were provided with a known number of capsules that storage in bottle, either cinnamon or placebo, for the following 4 weeks and then a new bottle for remaining weeks.

Each participant received the capsule bottles at baseline study day. At week 4, the participants returned the remaining capsules in the bottle and received a new capsule battles. At week 8, the participants returned the remaining capsules in the bottle. Each bottle was assessed by counting any remaining capsules in the bottles returned at 4 and 8 weeks.
Figure 7.1 Study design for the investigation of the effect of cinnamon over an 8-week study period.
7.3.5 Dietary intake

Food dietary intakes were recorded using information contained in the participants’ three-day diet diaries prior study date. The investigator explained the instructions and procedures to the participants who needed to complete the food diary. The completed food diary was analysed by DietPlan.

7.3.6 Blood sample collection

Fasting blood samples were taken at week zero, week four and week eight. They were analysed for fasting blood glucose levels, fasting insulin levels, the oral glucose tolerant test (0 and 8 week visits) and fasting lipid profiles, which included cholesterol (CHO), high density lipoproteins (HDL), triglycerides (TAG), and non-esterified fatty acids (NEFA). The blood samples were taken by trained phlebotomists who worked at the CIU. 2 ml of a fasting blood sample was drawn by a vacutainer safety lock blood collection set and single use holder. Blood was drawn into the vacutainer fluoride/oxalate grey tube (Becton Dickinson (BD) and company, Oxford, UK) for the determination of the plasma glucose. The same tube was used to determine glucose from the oral glucose tolerance test two hours after consuming a 75 g glucose drink. An additional 2 ml of a fasting blood sample was taken into lithium heparin vacutainer, (Becton Dickinson (BD) and company, Oxford, UK) to determine plasma insulin levels. A further 4 ml fasting blood sample was taken into a vacutainer containing dipotassium ethylene diamine tetraacetic acid (K₂EDTA) (Becton Dickinson (BD) and company, Oxford, UK) to determine plasma levels of CHO, HDL, TAG, and NEFA. Blood samples were immediately centrifuged at 1750 g for 10 min at 4 ºC to obtain the supernatant plasma, which was removed by pipette. Aliquots of the plasma were dispensed into appropriately labelled micro-centrifuge tubes (1.5 ml) and stored at -80 ºC until required for analysis.

7.3.7 Statistical analyses

SPSS software version 22 was utilised for the statistical analyses. A Kolmogorov-Smirnov analysis was performed to check for the normality of distribution of the data; checks were carried out for both normal and de-trended normal probability plots. The results of the analysis were represented as a mean ± standard error of the mean (SEM). Changes in fasting plasma glucose, insulin, HDL, LDL, CHO and NEFA levels were analysed using a repeated measures two-way ANOVA, whilst the baseline
variables of the two groups were compared with an unpaired \( t \)-test. In weeks four and eight, a paired \( t \)-test was used to compare the data with the baseline data for some of the variables. Relationships between the variables were examined using the Pearson correlation coefficient. Graphs were plotted through GraphPad Prism version 6 for Windows, with each graph showing the \( p \)-values for cinnamon treatment (T) and duration (D), and the relationship between them (D/T). Correlation coefficients were utilised in order to analyse the relationship between the changes in the different variables, where statistical significance was set at \( P < 0.05 \).

7.4 Results

7.4.1 Patient recruitment

As mentioned above (section 7.3.1) it was not possible to recruit the target number of 23 participants for each group; thus only 16 were recruited in total. A possible reason for this was that it was the summer holidays and some potential subjects had booked in advance for their holidays. Therefore, the study days were not convenient for them. In addition, the fact that the participants were required to be in a certain age range, as well as healthy, limited the number that could be included in the study. Furthermore, one of the participants’ BMI was 40, which did not comply with the inclusion criteria. Thus, the number of participants that were used in the statistical analyses was 15. Of the 15 participants taking part in the study, seven were randomly assigned to the placebo group, and eight to the cinnamon group.

7.4.2 Baseline characteristics

The baseline characteristics and anthropometric data of the participants in both groups (Table 7.1), show there were no significant differences in mean weight, height, age, BMI, hip circumference (HC) and waist circumference (WC). Significant differences were not observed between the fasting glucose and insulin levels of each group either. At the beginning of the study, both groups had normal, healthy levels of systolic and diastolic blood pressure, with no significant differences in diastolic blood pressure between the groups. However, the systolic blood pressure was higher in the group who were to receive cinnamon group (119.88 mm Hg) compared to the control group (108.00 mm Hg) \( (P = 0.03) \), which was perhaps just due to chance.
Table 7.1 The characteristics of the study participants at the baseline.

<table>
<thead>
<tr>
<th>Demographic</th>
<th>Cinnamon (n = 8) Mean ± SEM</th>
<th>Placebo (n = 7) Mean ± SEM</th>
<th>Unpaired t-test p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (m)</td>
<td>1.61 ± 0.01</td>
<td>1.65 ± 0.02</td>
<td>0.13</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>76.75 ± 2.7</td>
<td>77.29 ± 3.4</td>
<td>0.90</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>29.46 ± 1.0</td>
<td>28.34 ± 1.3</td>
<td>0.49</td>
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<tr>
<td>Waist circumference (cm)</td>
<td>94.50 ± 2.2</td>
<td>89.21 ± 2.2</td>
<td>0.11</td>
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<tr>
<td>Hip circumference (cm)</td>
<td>106.94 ± 2.3</td>
<td>108.00 ± 2.1</td>
<td>0.74</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>119.88 ± 3.1</td>
<td>108.00 ± 3.5</td>
<td>0.03*</td>
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<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>83.00 ± 3.2</td>
<td>74.71 ± 3.5</td>
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<tr>
<td>Heart rate (BPM)</td>
<td>67.50 ± 2.2</td>
<td>72.37 ± 3.9</td>
<td>0.28</td>
</tr>
<tr>
<td>Age (Years)</td>
<td>56.00 ± 1.3</td>
<td>53.00 ± 2.7</td>
<td>0.22</td>
</tr>
</tbody>
</table>

The clinical markers of the subjects at the baseline for both groups (Table 7.2) showed there was no significant difference in fasting glucose, fasting insulin, TAG, HDL, LDL and NEFA levels at the baseline. Also, in bone trabecular density mg/cm³ (BTrbDen1mgpercm³) there was no significant different between the groups. In terms of cholesterol, there was a significant different at the baseline between the cinnamon and control groups ($P = 0.016$), which was just due to chance. The fasting glucose levels of the cinnamon group was $5.80 ± 0.17$ mmol/l, and the control groups was $5.70±0.20$ mmol/l, which were marginally higher than the normal range and are considered pre-diabetic. Fasting insulin levels, cholesterol and LDL levels in the cinnamon group were slightly higher than the normal range, while the control group were in the normal range, whereas TAG and NEFA were in the normal range in both groups. HDL levels in both groups were desirable. The BTrbDen1mgpercm³ for both groups were in the normal range according to the literature (Grampp et al., 1997).
Table 7.2 The clinical markers of the participants at the baseline.

<table>
<thead>
<tr>
<th>Markers</th>
<th>Cinnamon (n = 8) Mean ± SEM</th>
<th>Placebo (n = 7) Mean ± SEM</th>
<th>Unpaired t-test p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting glucose mmol/l</td>
<td>5.80 ± 0.17</td>
<td>5.70 ± 0.20</td>
<td>0.638</td>
</tr>
<tr>
<td>Fasting insulin μU/ml</td>
<td>16.57 ± 2.09</td>
<td>11.51 ± 1.83</td>
<td>0.096</td>
</tr>
<tr>
<td>HOMA Beta-Cell Function (B%)</td>
<td>117.78 ± 9.11</td>
<td>97.67 ± 10.88</td>
<td>0.177</td>
</tr>
<tr>
<td>HOMA Sensitivity Insulin (S%)</td>
<td>53.46 ± 9.55</td>
<td>72.21 ± 7.52</td>
<td>0.155</td>
</tr>
<tr>
<td>HOMA Insulin Resistance (IR)</td>
<td>2.20 ± 0.28</td>
<td>1.53 ± 0.24</td>
<td>0.095</td>
</tr>
<tr>
<td>Triglyceride (TAG) mmol/l</td>
<td>0.96 ± 0.14</td>
<td>0.96 ± 0.09</td>
<td>0.984</td>
</tr>
<tr>
<td>Cholesterol (CHO) mmol/l</td>
<td>5.78 ± 0.21</td>
<td>4.84 ± 0.26</td>
<td>0.016*</td>
</tr>
<tr>
<td>HDL mmol/l</td>
<td>1.96 ± 0.20</td>
<td>1.76 ± 0.10</td>
<td>0.427</td>
</tr>
<tr>
<td>Non-esterified fatty acid (NEFA) mmol/l</td>
<td>0.82 ± 0.16</td>
<td>0.83 ± 0.25</td>
<td>0.962</td>
</tr>
<tr>
<td>LDL mmol/l</td>
<td>3.38 ± 0.22</td>
<td>2.63 ± 0.18</td>
<td>0.078</td>
</tr>
<tr>
<td>Bone Trabecular Density mg/cm³ (BTrbDen1mgpercm³)</td>
<td>182.70 ± 14.39</td>
<td>167.42 ± 11.74</td>
<td>0.434</td>
</tr>
</tbody>
</table>

7.4.3 Changes in anthropometric indices after supplementation

Four weeks into the study, there were significant reductions in weight ($P < 0.001$), WC ($P = 0.04$) and BMI ($P < 0.001$; Figure 7.2) in the cinnamon group, while no such reductions were observed in the control group (Figure 7.3). After eight weeks, these changes continued; in the cinnamon group, there were significant reductions in the participants’ weight ($P = 0.01$), BMI ($P = 0.01$), WC ($P = 0.001$) and HC ($P = 0.01$), and no change for the control group. However, there were small insignificant changes in the heart rate of the participants in the cinnamon group during 8 weeks (63.5 BPM) compared with baseline (67.50 BPM).

Overall, the group taking the C. cassia supplement lost about 2 kg in body weight each, while no changes were recorded in the placebo group after both four and eight weeks (Table 7.3). In terms of blood pressure (Table 7.3), there was a significant reduction in systolic blood pressure at weeks four and eight ($P = 0.04$, $P = 0.01$) and diastolic blood
pressure decreased significantly at week eight \((P = 0.02)\). There was no significant change observed in the control group.

**Figure 7.2:** Mean ± SEM of A: Weight, B: waist circumference and C: BMI for study participants as a whole at zero (baseline), 4 and 8 weeks of cinnamon supplementation. Significant difference from baseline, \(P<0.01^*, P<0.001^{**}\)
Figure 7.3: Mean ± SEM of A: Weight, B: waist circumference and C: BMI for study participants as a whole at zero (baseline), 4 and 8 weeks of control. Significant difference from baseline, $P<0.01^*$, $P<0.001^{**}$
### Table 7.3 Anthropometric measurements at weeks four and eight compared with the baseline. Values are means ± SEM.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Paired t. test p-value</th>
<th>Cinnamon</th>
<th>Paired t. test p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>Baseline</td>
<td>77.29 ± 3.4</td>
<td>76.75 ± 2.7</td>
<td></td>
</tr>
<tr>
<td>Change at 4 weeks</td>
<td>-0.14 ± 0.26</td>
<td>0.60</td>
<td>-1.00 ± 0.18</td>
<td>0.001*</td>
</tr>
<tr>
<td>Change at 8 weeks</td>
<td>-0.21 ± 0.40</td>
<td>0.62</td>
<td>-1.86 ± 0.55</td>
<td>0.01*</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>Baseline</td>
<td>28.34 ± 1.3</td>
<td>29.46 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>Change at 4 weeks</td>
<td>-0.05 ± 0.09</td>
<td>0.62</td>
<td>0.38 ± 0.06</td>
<td>0.001*</td>
</tr>
<tr>
<td>Change at 8 weeks</td>
<td>0.07 ± 0.14</td>
<td>0.64</td>
<td>-0.71 ± 0.20</td>
<td>0.01*</td>
</tr>
<tr>
<td><strong>Waist circumference (cm)</strong></td>
<td>Baseline</td>
<td>89.21 ± 2.2</td>
<td>94.50 ± 2.2</td>
<td></td>
</tr>
<tr>
<td>Change at 4 weeks</td>
<td>1.21 ± 1.57</td>
<td>0.47</td>
<td>-1.56 ± 0.60</td>
<td>0.04*</td>
</tr>
<tr>
<td>Change at 8 weeks</td>
<td>0.143 ± 1.01</td>
<td>0.89</td>
<td>-3.29 ± 0.73</td>
<td>0.004*</td>
</tr>
<tr>
<td><strong>Hip circumference (cm)</strong></td>
<td>Baseline</td>
<td>108.00 ± 2.1</td>
<td>106.94 ± 2.3</td>
<td></td>
</tr>
<tr>
<td>Change at 4 weeks</td>
<td>-0.35 ± 0.17</td>
<td>0.1</td>
<td>-0.65 ± 0.47</td>
<td>0.22</td>
</tr>
<tr>
<td>Change at 8 weeks</td>
<td>-0.64 ± 0.37</td>
<td>0.14</td>
<td>-2.57 ± 0.79</td>
<td>0.01*</td>
</tr>
<tr>
<td><strong>Systolic Blood pressure (mm Hg)</strong></td>
<td>Baseline</td>
<td>108.00 ± 3.5</td>
<td>119.88 ± 3.1</td>
<td></td>
</tr>
<tr>
<td>Change at 4 weeks</td>
<td>-4.00 ± 1.83</td>
<td>0.1</td>
<td>-5.13 ± 2.14</td>
<td>0.04*</td>
</tr>
<tr>
<td>Change at 8 weeks</td>
<td>-1.7 ± 2.94</td>
<td>0.58</td>
<td>-14.00 ± 3.82</td>
<td>0.01*</td>
</tr>
<tr>
<td><strong>Diastolic Blood pressure (mm Hg)</strong></td>
<td>Baseline</td>
<td>74.71 ± 3.5</td>
<td>83.00 ± 3.2</td>
<td></td>
</tr>
<tr>
<td>Change at 4 weeks</td>
<td>-1.86 ± 1.29</td>
<td>0.2</td>
<td>-3.25 ± 2.01</td>
<td>0.15</td>
</tr>
<tr>
<td>Change at 8 weeks</td>
<td>0.00 ± 1.83</td>
<td>1</td>
<td>-9.14 ± 3.06</td>
<td>0.02*</td>
</tr>
<tr>
<td><strong>Resting Heart Rate (BPM)</strong></td>
<td>Baseline</td>
<td>72.37 ± 3.9</td>
<td>67.50 ± 2.2</td>
<td></td>
</tr>
<tr>
<td>Change at 4 weeks</td>
<td>-0.80 ± 4.10</td>
<td>0.85</td>
<td>-3.38 ± 1.67</td>
<td>0.1</td>
</tr>
<tr>
<td>Change at 8 weeks</td>
<td>0.51 ± 3.96</td>
<td>0.9</td>
<td>-3.86 ± 1.94</td>
<td>0.09</td>
</tr>
</tbody>
</table>
7.4.4 Changes in plasma glucose concentrations

A fasting plasma glucose level is used as a marker to diagnose a pre-diabetic state and diabetes. Pre-diabetes, obesity and insulin resistance are all considered as risk factors for the development of CVD. Studies in vitro and in vivo have shown that herbal medicines, such as cinnamon, cloves and tea, may help to maintain normal blood glucose levels and delay the development of diabetes (Broadhurst et al., 2000). Therefore, this study measured the effect of cinnamon supplementation on fasting glucose levels.

The fasting glucose levels in the cinnamon group and the control group were within the normal range at baseline (Table 7.2) and there was no significant difference between the groups. There was no significant effect (Figure 7.4) of consuming 5 g of a cinnamon supplement on fasting glucose levels after four or eight weeks, and there was no change in the control group either.

In terms of the results from the oral glucose tolerance tests (OGTT), which were assessed at the baseline and after eight weeks, in cinnamon and control groups the baseline values were 5.5 ± 0.02 and 5.6 ± 0.42 respectively. After 8 weeks, the OGTT of cinnamon and control were 5.9 ± 0.04, 5.4 ± 0.6. The results were presented as Mean ± SEM. The OGTT levels of both groups were within the normal change at baseline and after 8 weeks. There was insufficient data produced for a statistical analysis (ANOVA) to be performed. However, based on the results the data shows that the OGTT levels were approximately similar at baseline and week 8 in both groups.
7.4.5 Changes in plasma fasting insulin levels

The fasting insulin levels at baseline (Table 7.2) in the cinnamon group were higher than normal range (16.43 µU/L), whilst in the control group the levels within the normal range (11.10 µU/L), according to the insulin assay protocol (Millipore Insulin kit, HI-14K) which states the normal range of fasting plasma insulin is 5-15 µU/l. At the baseline there were no significant differences in the fasting insulin level between the groups. In the cinnamon group (Figure 7.5) the fasting insulin level slightly reduced from 16.43 µU/L at the baseline to 14.64 µU/L at week four and to 13.05 µU/L at week eight, however this reduction did not achieve statistical significance ($P = 0.340$, $P = 0.075$, respectively). In the control group the fasting plasma insulin concentration increased slightly at weeks four and eight (13.47 µU/L and 12.36 µU/L, versus baseline, respectively), but again this was not a statistically significant difference. There was a positive correlation between fasting insulin and systolic blood pressure ($P = 0.030$, $R = 0.561$). Reducing systolic blood pressure was associated with decreasing...
in fasting insulin levels. Overall these results suggest the cinnamon was having a small effect on insulin levels, which could be linked to the weight loss and reducing blood pressure observed. These results may have been significant if the sample size was larger.

Figure 7.5: Fasting insulin concentrations at baseline, weeks four and eight of control and cinnamon groups. Fasting insulin levels were determined in each participant (n = 15) at week 0, 4 and 8. Individual values are displayed with Mean ± SEM. ANOVA two-way analysis of variance, T treatment effect, D duration effect and D/T treatment and duration interaction effect.

7.4.6 Changes in Homeostasis Model Assessment (HOMA)

The homeostasis model assessment has been widely used for the estimation of insulin resistance (HOMA-IR). The HOMA-IR model was originally produced by Matthews et al. (1985) and is calculated by multiplying the fasting plasma insulin (FPI) level by the fasting plasma glucose (FPG) level, then dividing that value by 22.5 (Matthews et al., 1985). Levy et al. (1998) have update HOMA-2. There is an updated version of this model, HOMA-2, which uses computer calculations to estimate insulin resistance (IR), insulin sensitivity (%S) and β-cell function (%B) (Wallace et al., 2004). In this study the HOMA-2 calculator was downloaded from the University of Oxford (Diabetes Trails
Unit, 2007) and was used to estimate IR, %B and %S, based on the fasting glucose and insulin levels. There is no approximation formula for HOMA-2 and there is also no normal range for the measures, as originally 100 % was meant to represent a normal person. A higher percentage of %S indicates a greater insulin sensitivity and lower ones less sensitivity. Once HOMA-2 insulin resistance (IR) is greater than 1 or %S less than 100, this means there is a greater insulin resistance. Insulin sensitivity and beta cell function have an inverse relationship.

7.4.6.1. HOMA insulin resistance index (HOMA-IR)

There was no significant difference for insulin resistance between the cinnamon and control groups at the baseline ($P = 0.095$). The insulin resistance data for both groups at the baseline showed they have a higher insulin resistance 2.20 and 1.53, respectively than expected (Table 7.2). In the cinnamon group (Figure 7.6) the insulin resistance decreased after four and eight weeks of consuming 5 g of cinnamon (2.01 and 1.82; $P = 0.364$, $P = 0.080$, respectively), though, this reduction was not significant. In the control group, the insulin resistance value increased slightly at weeks four and eight (1.77 and 1.66, respectively) compared with the baseline (1.53), but again this was not significant. These results agree with the fasting insulin levels which also showed a slight reduction and could be linked to the weight loss observed. There was a significant correlation between insulin resistance and systolic blood pressure ($P = 0.024$, $R = 0.579$) that means reducing blood pressure associated with reduced insulin resistance. This could be linked to lower blood pressure observed. These results may have been significant if the sample size was larger.
Figure 7.6: Insulin resistance at baseline, weeks four and eight of control and cinnamon groups. Insulin resistance were calculated in each participant (n = 15) at week 0, 4 and 8. Individual values are displayed with Mean ± SEM. ANOVA two-way analysis of variance, T treatment effect, D duration effect and D/T treatment and duration interaction effect.

7.4.6.2 HOMA β-cell function (HOMA-B %)

At the baseline (Table 7.2) there was no significant difference between the cinnamon and control groups (P = 0.177). The results (Figure 7.7) show there was no significant effect of the cinnamon in terms of the β-cell function index (P = 0.284). The function of the β-cells were somewhat higher than normal (Figure 7.7) in the cinnamon group at weeks zero, four and eight (117.78 %, 112.20 % and 107.90 %, respectively), and the β-cell function was reduced slightly over time so it became closer to the normal functional levels. In the control group however, β-cell function was within the normal range of function at weeks zero, four and eight (97.67 %, 102.46 % and 101.80 % respectively). These results follow the same pattern as the insulin level and the insulin resistance, which suggests the treatment is having a small effect in improving the health of the participants.
Figure 7.7: B cell function at baseline, weeks four and eight of control and cinnamon. B cell function was calculated in each participant ($n = 15$) at week 0, 4 and 8. Individual values are displayed with Mean ± SEM. ANOVA two-way analysis of variance treatment effect, D duration effect and D/T treatment and duration interaction effect.

7.4.6.3 HOMA insulin sensitivity (HOMA- S %)

There was no significant difference in insulin sensitivity at baseline (Table 7.2) between the two groups ($P = 0.155$). There was also no significant change in insulin sensitivity (%) after treatment ($P = 0.142$) (Figure 7.8), but it did increase slightly after consuming cinnamon at weeks four and eight (63.87 % and 61.75 %; $P = 0.070$, $P = 0.080$, respectively) compared with the baseline (53.46 %). In contrast, the insulin sensitivity in the control group decreased slightly at weeks four and eight (62.19 % and 62.33 %, respectively) compared to the baseline (72.21 %). This small change agrees with the small difference seen in β- cell function as the two are linked, and this could be due to the weight loss observed in the treatment group. The small sample size may be the limiting factor which is stopping these results from being significant. The data in this graph are quite disperse due to differences in the participants in this study. This variation would possibly be reduced if there were a larger sample size.
Figure 7.8: Insulin sensitivity at baseline, weeks four and eight of control and cinnamon groups. Insulin sensitivity was calculated in each participant (n = 15) at week 0, 4 and 8. Individual values are displayed with Mean ± SEM. ANOVA two-way analysis of variance, T treatment effect, D duration effect and D/T treatment and duration interaction effect.

7.4.7 Changes in plasma fasting lipid profile levels

7.4.7.1 Changes in plasma triglyceride TAG

At baseline (Table 7.2) there was no significant difference in the plasma TAG levels between the control and cinnamon groups (P = 0.984). There was no effect of the cinnamon treatment (Figure 7.9) on plasma TAG concentrations during the eight weeks (P = 0.584). However, there was a significant effect of duration (P = 0.046); the plasma TAG concentration increased slightly at week four in the cinnamon and control groups (1.12 mmol/l and 1.02 mmol/l, respectively), and then decreased slightly at week eight, becoming similar to the baseline levels for both the groups (0.99 mmol/l cinnamon, and 0.95 mmol/l control). All the plasma TAG levels in both groups were within normal range. This change over time may be as a result the spread of the data rather than an actual change in the groups. Overall these results show the variability
in TAG levels over time, and these may be linked to changes in the participants' diets over the course of the study.

![Graph showing triglyceride concentrations over time for placebo and cinnamon groups.]

**Figure 7.9:** Triglyceride concentrations at baseline, weeks 4 and 8 of control and cinnamon groups. Triglyceride levels were determined in each participant \((n = 15)\) at week 0, 4 and 8. Individual values are displayed with Mean ± SEM. ANOVA two-way analysis of variance, **T** treatment effect, **D** duration effect and **D/T** treatment and duration interaction effect.

### 7.4.7.2 Changes in plasma cholesterol (CHO)

At the baseline (Table 7.2) there was a significant difference in the CHO levels between the control and cinnamon groups \((P = 0.016)\). This is possibly because the cinnamon group had a slightly higher BMI, waist circumference, and hip circumference, which would mean they had a higher % body fat which could affect the CHO concentration. The cholesterol concentration (Figure 7.10) in the cinnamon group at weeks zero, four and eight were slightly higher \((5.78 \text{ mmol/l}, 5.75 \text{ mmol/l}, 5.83 \text{ mmol/l}, \text{ respectively})\) than in the control groups \((4.84 \text{ mmol/l}, 4.64 \text{ mmol/l}, 4.60 \text{ mmol/l}, \text{ respectively})\). Over time, there was no significant effect of the cinnamon treatment on CHO concentrations \((P = 0.584, P = 0.046, P = 0.257)\)
Overall this suggests that, despite the associated weight loss, the cinnamon treatment did not affect the concentrations of circulating CHO.

Figure 7.10: Cholesterol concentrations at baseline, weeks 4 and 8 of control and cinnamon groups. Cholesterol levels were determined in each participant \((n = 15)\) at week 0, 4 and 8. Individual values are displayed with Mean ± SEM. ANOVA two-way analysis of variance, \(T\) treatment effect, \(D\) duration effect and \(D/T\) treatment and duration interaction effect.

7.4.7.3 Changes in plasma high density lipoprotein (HDL)

There was no significant difference in the HDL concentrations between the groups at the baseline \((P = 0.427)\) (Table 7.2), but the cinnamon group was slightly higher. The HDL levels decreased slightly in the control group at weeks four and eight (Figure 7.11) \((1.69 \text{ mmol/l} \text{ and } 1.62 \text{ mmol/l}, \text{ respectively})\) compared with the baseline \((1.76 \text{ mmol/l})\). In the cinnamon group the HDL concentrations increased slightly by week eight \((2.01 \text{ mmol/l})\) compared to the baseline \((1.96 \text{ mmol/l})\), but neither of these changes reached statistical significance. Overall these results show that the cinnamon treatment caused small but insignificant changes to the HDL levels that could correlate with the weight loss observed.
Figure 7.11: HDL concentrations at baseline, weeks 4 and 8 of control and cinnamon groups. HDL were determined in each participant \((n=15)\) at week 0, 4 and 8. Individual values are displayed with Mean ± SEM. ANOVA two-way analysis of variance, T treatment effect, D duration effect and D/T treatment and duration interaction effect.

7.4.7.4 Changes in plasma low density lipoprotein (LDL)

The LDL value was calculated using Friedwald et al.’s (1972) formula:

\[
\text{LDL} = \text{Total Cholesterol} - (\text{HDL} - (\text{TAG}/2.2)) \text{ mmol/l}
\]

At the baseline (Table 7.2) there was no significant difference in the LDL levels between the control and cinnamon groups \((P = 0.78)\). The LDL value in the cinnamon group at weeks four and eight (Figure 7.12) were slightly higher than in the control group \((4.34 \text{ mmol/l vs } 3.41 \text{ mmol/l} \text{ and } 4.26 \text{ mmol/l vs } 3.41 \text{ mmol/l}, \text{ respectively})\), but the differences were not statistically significant. In the cinnamon group, the LDL: HDL ratio after eight weeks was 1.7, which was similar to the baseline (1.7) and week four (1.7). In the control group, the ratio of LDL: HDL at the baseline was 1.5 and approximately remained the same after eight weeks (1.6). Overall these results show that the cinnamon treatment had no significant effect on the calculated LDL levels.
Figure 7.12: LDL concentrations at baseline, weeks 4 and 8 of control and cinnamon groups. LDL were calculated in each participant (n = 15) at week 0, 4 and 8. Individual values are displayed with Mean ± SEM. ANOVA two-way analysis of variance, T treatment effect, D duration effect and D/T treatment and duration interaction effect.

7.4.7.5 Changes in plasma non-esterified fatty acids (NEFA)

There were no significant differences in the NEFA levels at the baseline (P = 0.962) (Table 7.2). However, there was a significant effect of the treatment (P = 0.017) (Figure 7.13), which showed consuming 5 g of a cinnamon supplement reduced the NEFA concentrations significantly. At the baseline, NEFA levels in the cinnamon and control groups were similar (0.82 mmol/l and 0.83 mmol/l, respectively), whereas after four and eight weeks, the NEFA concentrations in the cinnamon group decreased significantly to 0.48 mmol/l and 0.57 mmol/l, respectively. There was also a slight decrease in the NEFA levels for the control at weeks for and eight, but the reduction was not as large. This decrease in NEFA shows that the cinnamon treatment is having an effect. There was a positive correlation of NEFA with fasting insulin and insulin resistance (P = 0.030, R = 0.560; P = 0.028, R = 0.566, respectively). These results mean that reducing NEFA levels was associated with a reduction in fasting insulin and insulin resistance, which was observed in our data. Overall these results show that the
cinnamon treatment has significant effect on NEFA levels which fits with the slight reduction that was observed in fasting insulin and insulin resistance values.

![Graph showing NEFA concentrations at baseline, weeks 4 and 8 of control and cinnamon groups.](image)

**Figure 7.13:** NEFA concentrations at baseline, weeks 4 and 8 of control and cinnamon groups. NEFA were determined in each participant (n = 15) at week 0, 4 and 8. Individual values are displayed with Mean ± SEM. ANOVA two-way analysis of variance treatment effect, D duration effect and D/T treatment and duration interaction effect.

### 7.4.8 Macronutrient composition calculated from the diet diaries

Three-day diet diaries were collected from each participant prior to each study day trial. The (Table 7.4) macronutrient intake of the participants in both groups at the baseline shows there were no significant difference in the total energy intake, or in the intake of proteins, carbohydrates, total sugars, total fat, saturated fatty acids and fibre at the baseline.
Table 7.4 The macronutrient intake data from the three day diaries prior to the study day at the baseline. Data displayed as Mean ± SEM for the three days.

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Control (n = 7) Mean ± SEM</th>
<th>Cinnamon (n = 8) Mean ± SEM</th>
<th>Unpaired t-test (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
<td>1804.86 ± 175.53</td>
<td>1753.12 ± 117.89</td>
<td>0.806</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>70.84 ± 6.63</td>
<td>80.63 ± 4.39</td>
<td>0.229</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>200.27 ± 18.04</td>
<td>173.73 ± 14.90</td>
<td>0.273</td>
</tr>
<tr>
<td>Total Sugars (g)</td>
<td>79.13 ± 4.56</td>
<td>76.83 ± 7.67</td>
<td>0.809</td>
</tr>
<tr>
<td>Total Fat (g)</td>
<td>76.99 ± 10.42</td>
<td>77.47 ± 8.00</td>
<td>0.970</td>
</tr>
<tr>
<td>Saturated Fatty Acids (g)</td>
<td>27.93 ± 4.02</td>
<td>24.26 ± 2.35</td>
<td>0.431</td>
</tr>
<tr>
<td>Fibre (g)</td>
<td>17.97 ± 3.48</td>
<td>17.31 ± 1.81</td>
<td>0.864</td>
</tr>
</tbody>
</table>

7.4.8.1 Changes in macronutrient composition between the groups over eight weeks

There was no significant change regarding the total energy intake, or the intake in proteins, carbohydrates, total sugars, total fat, saturated fatty acids and fibre after the intervention (Table 7.5 and Table 7.6) compared with the baseline (Table 7.4) in both groups. The results show that at week four (Table 7.5) in the cinnamon group there was a decrease in carbohydrate (28.36 g), sugar (8.33 g) and energy intake (44.75 kcal) compared with the baseline, though this was not a statistically significant difference. Furthermore, in the control group at week four the carbohydrate and sugar intake decreased (14.24 g and 7.59 g, respectively) compared with the baseline. At week eight (Table 7.6) there were no significant changes in either group for all macronutrients. Overall this suggests that being in the study did not have a significant effect on the diet of the participants, at least those that were recorded.
### Table 7.5
The macronutrient intake changes compared to baseline calculated from the three day diaries prior to the study day at week four.

<table>
<thead>
<tr>
<th>Macronutrients</th>
<th>Control (n = 7) Mean ± SEM</th>
<th>Paired t-test (p-value)</th>
<th>Cinnamon (n = 8) Mean ± SEM</th>
<th>Paired t-test (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
<td>28.86 ± 101.14</td>
<td>0.785</td>
<td>-44.75 ± 241.57</td>
<td>0.858</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>5.06 ± 5.90</td>
<td>0.424</td>
<td>0.83 ± 6.09</td>
<td>0.896</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>-14.24 ± 17.13</td>
<td>0.438</td>
<td>-28.36 ± 27.32</td>
<td>0.334</td>
</tr>
<tr>
<td>Total Sugars (g)</td>
<td>-7.59 ± 6.27</td>
<td>0.272</td>
<td>-8.33 ± 12.69</td>
<td>0.533</td>
</tr>
<tr>
<td>Total Fat (g)</td>
<td>7.57 ± 3.78</td>
<td>0.092</td>
<td>2.75 ± 11.61</td>
<td>0.820</td>
</tr>
<tr>
<td>Saturated Fatty Acids (g)</td>
<td>4.83 ± 2.80</td>
<td>0.135</td>
<td>0.49 ± 3.92</td>
<td>0.905</td>
</tr>
<tr>
<td>Total Dietary Fibre (g)</td>
<td>0.39 ± 2.17</td>
<td>0.865</td>
<td>-2.01 ± 1.60</td>
<td>0.248</td>
</tr>
</tbody>
</table>

### Table 7.6
The macronutrient intake changes compared to baseline calculated from the three day diaries prior to the study day at week eight.

<table>
<thead>
<tr>
<th>Macronutrients</th>
<th>Control (n = 7) Mean ± SEM</th>
<th>Paired t-test (p-value)</th>
<th>Cinnamon (n = 8) Mean ± SEM</th>
<th>Paired t-test (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
<td>-31.14</td>
<td>129.50</td>
<td>-1.71</td>
<td>132.35</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>-2.94</td>
<td>5.38</td>
<td>2.49</td>
<td>4.12</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>-15.11</td>
<td>13.02</td>
<td>-6.69</td>
<td>9.13</td>
</tr>
<tr>
<td>Total Sugars (g)</td>
<td>-10.39</td>
<td>8.40</td>
<td>0.20</td>
<td>9.43</td>
</tr>
<tr>
<td>Total Fat (g)</td>
<td>3.17</td>
<td>7.42</td>
<td>-0.87</td>
<td>9.22</td>
</tr>
<tr>
<td>Saturated Fatty Acids (g)</td>
<td>3.23</td>
<td>3.98</td>
<td>-2.14</td>
<td>2.50</td>
</tr>
<tr>
<td>Total Dietary Fibre (g)</td>
<td>1.71</td>
<td>2.28</td>
<td>-1.47</td>
<td>0.99</td>
</tr>
</tbody>
</table>
7.5 Discussion

Some studies have suggested that cinnamon has strong antioxidant, antibacterial, anti-inflammatory, and anti-cancer and anti-viral properties, and therefore can be used in the treatment of common colds, CVD, and chronic gastrointestinal complaints (Verspohl et al., 2005; Kim et al., 2006; Molania et al., 2012; Hong et al., 2012). This study has shown that ingestion of 5 g of a C. cassia supplement for eight weeks reduced weight significantly \( (P = 0.01) \). Moreover, a significant reduction also occurred in the BMI and waist circumference of the participants in the treatment group when compared to the control. The total energy intake at the baseline in cinnamon and control groups were 1753.13 kcal, 1804.86 kcal, respectively. The total energy intake for both groups was lower than recommended dietary allowance (RDA) recommendation of energy (1900 kcal) by 7.7 % in cinnamon group and 4.86 % in control. The mean total carbohydrate consumed was 200.27 g in cinnamon group, which was 70 g the above RDA recommendation of 130 g, whereas the carbohydrate consumed in control (173.73 g) was slightly lower but also 43.73 g higher then RDA recommendation, which could influence the ability of the participants to lose weight. The potential change in weight based on energy intake and energy expenditure in the cinnamon and control groups was calculated using the details gathered in this study. It was estimated that the cinnamon and control participants had 30 min light exercise and 30 min standing and shopping per day. According to their age, weight, height and exercise amount, the mean total energy expenditure was 2534 kcal per day (http://www.health-calc.com/diet/energy-expenditure-advanced). The estimated weight loss in cinnamon group was between –0.78 kg, -0.74 kg per week. Whereas in control group the estimated weight loss was between -0.69 kg -0.72 kg per week. Therefore, the energy intake in both groups should help them to reduce their weight. However, the significant reduction was observed in cinnamon group only.

There are many mechanisms that may explain the weight loss, for example analysis of the food intake for the cinnamon group over four weeks showed a slight reduction in the total energy intake (from 1753.13 kcal to 1708.37 kcal), which, as mentioned above, is partially responsible for the loss of one kilogram observed after four weeks, and the further loss over the following four weeks to give a total loss of about two kilograms. It is possible that cinnamon affected the satiety and gastric emptying rate,
though this study did not determine either of these. Other studies have confirmed that adding 6 g of cinnamon to 300 g of rice pudding significantly reduced the postprandial blood glucose concentration and delayed gastric emptying rate in 14 healthy subjects, but these researchers found that the cinnamon did not affect satiety (Hlebowicz et al., 2007). It may be that the consumption of 5 g of cinnamon in this study delays the gastric emptying rate, and thus these participants consumed slightly less in total energy, which leads to a reduction in weight. In a different study it was shown that adding 1 or 3 g of *C. cassia* to 300 g of rice pudding did not affect the gastric emptying rate, glucose or satiety in 15 healthy subjects, whereas 3 g of *C. cassia* significantly lowered the insulin response at 60 min and the area under the curve (AUC) at 120 min (Hlebowicz et al., 2009). Overall these studies show that the concentration of *C. cassia* selected can have a large effect on the changes observed within the study.

Controlling the insulin concentration has been shown to play an important role in managing an individual’s weight (Woods et al., 1985). Hlebowicz et al. (2009) showed that 3 g of cinnamon added to 300 g of rice pudding significantly reduced postprandial insulin response in healthy individuals, though this did not change the blood glucose concentration in the healthy subjects. The effect of cinnamon in Hlebowicz et al. (2007) and Hlebowicz et al. (2009) studies were acute studies and therefore they did not examine the long term effect of consuming cinnamon, whereas our study investigated the long term effects (eight weeks) and our results showed an insignificant reduction in the plasma fasting insulin levels.

Studies *in vitro* have shown that cinnamon extract stimulated insulin receptors by activating insulin receptor kinase and insulin sensitivity (Imparl-Radosevich et al., 2000). Further evidence of the effect of cinnamon comes from studies *in vivo* that showed oral administration of a cinnamon extract (30 and 300 mg/kg BW) for three weeks enhanced glucose utilization in rats, through stimulating insulin tyrosine phosphorylation of the insulin receptor-β and insulin receptor substrate-1 in skeletal muscle (Qin et al., 2003). This effect might be due to the fact that the administration cinnamon dose was very large (30 and 300 mg/kg BW). While in this study, the dose that participants consumed was 5 g per day, which was about 64.93 mg/kg body weight. Also, 300 mg of cinnamon per kg of body weight of rats is equivalent to 23 g of cinnamon in human. Therefore, in the animal study the large cinnamon dose may be
enough to enhance glucose uptake through stimulating insulin to bind with insulin receptors, whereas in our study the dose was not large enough to see this effect.

Our findings showed that consuming 5 g of cinnamon for eight weeks led to a lower plasma fasting insulin level, from 16.43 µU/l at baseline to 13.05 µU/l at week eight, although this was not statistically significant. These results suggest that cinnamon may help reduce the demand for insulin. In addition, insulin resistance in the cinnamon group reduced from 2.20 at baseline to 1.82 at week eight, whereas in the control group there was a slight increase in this parameter. However, these changes did not reach a statistically significance level ($P = 0.690$), possibly due to the small sample size. In our data there was a positive correlation between systolic blood pressure and insulin resistance and fasting insulin ($P = 0.024$, $R = 0.579$; $P = 0.030$; $R = 0.561$). These results suggest that cinnamon has a small effect on insulin levels, which could be linked to the significant reduction in systolic blood pressure that was observed. In addition, it maybe that cinnamon improves the insulin signalling pathway, which would cause a reduction in the insulin resistance. This correlation was confirmed also by Baron et al. (1993) that hypertension is associated with insulin resistance.

There is evidence to suggest that elevated plasma NEFA concentrations are associated with insulin resistance (Karpe et al., 2011), which correlates with our results that showed that taking 5 g of *C. cassia* for eight weeks significantly reduced NEFA ($P = 0.017$). According to our knowledge this study was the first study looking for the effect of cinnamon on NEFA levels. In this study there was a positive correlation of NEFA with fasting insulin and insulin resistance ($P = 0.030$, $R = 0.560$; $P = 0.028$, $R = 0.566$, respectively). Our results confirmed that a slight reduction in fasting insulin levels and insulin resistance were associated with a significant reduction in NEFA levels due to the cinnamon treatment. In addition, elevated plasma NEFA concentrations might be a marker for MetS, type 2 diabetes and CVD (Frayn and Williams, 1996). Therefore, the use of a 5 g cinnamon supplement that can significantly reduce the NEFA levels might improve overall metabolism and reduce the risk in the incidence of type 2 diabetes and CVD, without any of the side effects associated with the usual medications. Furthermore, elevated NEFA concentrations can impair endothelial function and raise blood pressure (Steinberg et al., 2000; Florian et al., 2010). This, interestingly, is consistent with our findings (Chapter six) where ingesting 5 g of a cinnamon supplement slightly improved the expression of some adhesion molecules.
(sVCAM, sICAM, sESEL, sPSEL, sLSEL) and significantly reduced blood pressure. Thus, our data shows that cinnamon significantly reduced NEFA concentrations which may be linked to the reduced weight, blood pressure and improved fasting insulin levels and improving endothelial function. There was, however, no difference in the ratio of LDL: HDL after eight weeks of intervention.

Cinnamon contains an active biological compounds that might imitate insulin’s properties and improve glucose uptake in the body, for example *C. cassia* is rich in type-A procyanidins (Chapter 3). This finding is consistent with previous studies, as *in vitro* studies have highlighted that type-A procyanidin isolated from cinnamon enhances insulin receptors and increases insulin sensitivity (Anderson et al., 2004; Lu et al., 2011). This is likely to be one of the mechanisms of metformin with which it acts to produce satiety and weight loss (Lee and Morley., 1998). Our outcome showed consuming 5 g cinnamon for eight weeks caused a slightly reduced fasting insulin, however, this change in insulin did not lead to a change in fasting blood glucose. This, however, might be due to the low number of study subjects, but it is supported by the work of Wickenberg et al. (2012) who found that ingesting 6 g of Ceylon cinnamon did not have an effect on glucose levels and insulin response on participants with impaired glucose tolerance. The participants in our study were considered to be pre-diabetic because their fasting glucose was over 5.6 mmol/m, and in addition, their fasting insulin levels were high at the baseline, which indicated there was some insulin resistance.

Moreover, cinnamon has been used in studies to treat people with type 2 diabetes. Khan et al. (2003) showed that ingesting 1, 3 and 6 g of *C. cassia* powder every day for 40 days lowered fasting glucose, TAG, LDL and total cholesterol of patients with type 2 diabetes. Nevertheless, consuming 112 mg of aqueous *C. cassia* extract in capsules, which corresponded to 3 g of cinnamon per day, for four months in participants with type 2 diabetes, reduced fasting plasma glucose but had no effect on CHO, LDL, HDL and TAG levels (Mang et al., 2006). In contrast, Blevins et al. (2007) found that ingesting 1 g of *C. cassia* every day for three months did not improve fasting glucose, or lipid and insulin concentrations in people with type 2 diabetes, but it is possible that this dose was too small to see an effect. Furthermore, there was no improvement seen in fasting plasma glucose levels, insulin levels, TAG, LDL, CHO, HDL, or insulin resistance in overweight postmenopausal women with type 2 diabetes consuming 1.5 g of *C. cassia* every day for six weeks (Vanschoonbeek et al., 2006).
Further support for a positive effect of consuming low doses of a cinnamon supplement comes from a study by Akilen et al. (2010). In their study, 58 poorly controlled type 2 diabetic participants consumed 2 g of *C. cassia* supplement every day for 12 weeks. Fasting glucose, haemoglobin A1c (HbA1c), systolic and diastolic blood pressures were reduced significantly in the cinnamon group compared with the control group. Moreover, their BMI and waist circumference decreased, although this was not significant. There were also no significant differences in lipid profiles of total cholesterol, triglycerides, HDL and LDL cholesterol. The results of Akilen et al. (2010) study was consistent with our findings for the lipid profile (total cholesterol, HDL and LDL cholesterols), which showed no effect of cinnamon on the lipid profiles. Overall our outcomes show that the cinnamon treatment has no significant effect on LDL, CHO and HDL levels, which did not fit with the weight loss observed. In our finding there was no correlation between weight and LDL, CHO and HDL (*P* = 0.398, *P* = 0.268, *P* = 0.195, respectively). In systemic meta-analysis review that found the anti-obesity drugs were reduced 3.13 kg compared with placebo, however, this did not significantly reduce LDL, CHO and fasting glucose (Zhou et al., 2012). Thus, decreasing LDL, CHO and fasting glucose not necessarily associated with losing weight.

5 g of *C. cassia* for eight weeks significantly reduced NEFA (*P* = 0.017), and according to our knowledge this study was the first investigation looking for the effect of cinnamon on NEFA. The reduction in the fasting glucose levels in the subjects that were involved in the Akilen et al. (2010) study was due to the fact that the subjects had poorly controlled glucose levels, whilst our study participants were healthy. Therefore, the effects of cinnamon on the normal fasting glucose level, which was 5.8 mmol/l in the cinnamon group, may be minimalized, although we used 5 g of cinnamon for eight weeks. Ziegenfuss et al. (2006) evaluated the highest cinnamon dose (10 g) on fasting plasma glucose levels among pre-diabetics. They used 500 mg of Cinnulin PF® which is equivalent to approximately 10 g of whole cinnamon powder (i.e. 20:1 extract) and contains at least 1 % doubly-linked polyphenol type-A polymers. The baseline fasting plasma glucose was 6.46 mmol/l, and after 12 weeks of consuming 10 g equivalent of cinnamon, the fasting glucose and systolic blood pressure were significantly reduced. This effect on fasting glucose and systolic blood pressure might be due to their baseline levels being higher than normal, and therefore there was more scope for an effect to be seen in these subjects. Also, the cinnamon supplement was an extract of cinnamon...
and not cinnamon powder, which was equivalent to 10 g of cinnamon powder, which is a high dose. However, in our study we did not find an effect of using 5 g of cinnamon in the fasting glucose results. This could be explained by the fact that our participants were healthy and their baseline fasting glucose levels were normal and lower than Ziegenfuss et al. (2006) participants. It is clear that there are conflicting findings on the effect of cinnamon, which may depend on the cinnamon dose, classification of the cinnamon used, the subject’s health status and the level of the baseline markers. In Khan et al. (2003) study, the participants had poorly controlled glucose levels and they did not receive anti-diabetic therapeutic treatments. In their study the positive effect of cinnamon was clear with high doses of cinnamon, which were 6 g, whilst it seems there was no effect using a low dose of cinnamon for the duration of the study.

Interestingly, *C. cassia* has been shown to be more effective than *C. zeylanicum* in *vivo* studies. For example, Wickenberg et al. (2012) used a high dose of *C. zeylanicum* (6 g), but this treatment did not lead to a change in the fasting glucose, lipid or insulin concentrations, which agrees with our findings that the concentrations of polyphenols in these two types of cinnamon are different (Chapter 3), which could explain why the responses were not the same.

The main cause of obesity and being overweight is an imbalance between energy intake and energy expenditure, therefore weight loss can be achieved through a reduction in food intake or increasing energy expenditure (Hursel and Westerterp-Plantenga., 2010). Stimulation of fat oxidation and thermogenesis in the body is one approach in treating obesity and the management of an individual’s weight; the sympathetic nervous system (SNS) regulates energy expenditure and it is involved in thermogenesis, which can lead to an increase in the use of ATP, or to an increase in the rate of mitochondrial oxidation that increases heat production, which expends excess energy as heat (Westerterp-Plantenga et al., 2006). The interest in the thermogenic effects of natural herbal compounds, which affect the metabolism via satiety and increased energy expenditure, such as spices and herbs, has increased, especially because these ingredients do not contain any energy themselves (Henry and Emery., 1989; Dulloo, 1993; Hursel and Westerterp-Plantenga., 2010).
The anti-obesity thermogenic agents have cardiovascular effects such as increased hypertension and heart rate (Dulloo, 1999). Therefore, the interest in plants and herbs, such as ginger and green tea, that are not accompanied by cardiovascular effects has increased; consuming a ginger beverage affects thermogenic and increases satiety significantly, which suggests the potential role of ginger in weight management (Mansour et al., 2012). Studies have also shown that consuming 525 mg of green tea extract in capsular form, containing catechin polyphenol and caffeine, for 6 weeks significantly increases energy expenditure (4 %), and carbohydrate and fat oxidation in a 24 hour period, but does not increase the heart rate (Dulloo, 1999). Catechins in the green tea inhibit the enzyme catechol O-methyltransferase (COMT) that degrades catecholic compounds (Hursel and Westerterp-Plantenga., 2010). Catechin is one of the polyphenols found in cinnamon that was identified by LC-MS (Shan et al., 2007). This suggests that cinnamon catechins may play a similar role to green tea catechin, and inhibit catechol O-methyltransferase enzyme. Furthermore, procyanidins are metabolised by gut flora and broken down in the gut to catechins (Forester and Waterhouse, 2009), which may act in a similar way to green tea catechins.

The LC-MS and HPLC analysis of cinnamon samples in this thesis confirmed that cinnamon is also rich in catechin polyphenols (Chapter 3). Therefore, it might be that cinnamon catechin polyphenols increased the energy expenditure and fat oxidation in the cinnamon participants, which led to a significant reduction in their weight. However, there was no effect on heart rate in the cinnamon group, and in this respect, the consumption of cinnamon is distinct from anti-obesity thermogenic agents, which increase the heart rate and can have cardiovascular effects. Unfortunately, there have been no studies published on cinnamon in relation to energy expenditure and fat oxidation. Our data showed that there is an effect of cinnamon on lipid profiles as NEFA was reduced significantly after consuming 5 g of cinnamon. This might be confirmation that cinnamon has a positive effect on fat metabolism, although the food diary analysis showed that there was no significant reduction in energy intake, but the energy intake, carbohydrate and fat of the cinnamon participants did decrease slightly during the cinnamon treatment.

Despite the strengths of this RCT study design, there are some limitations to it, the key one being the small sample size that was obtained, which reduced the significance of the findings of the study. Within this study the counting of the capsules remaining in
the supplied bottles at 4 and 8 weeks in both groups, suggests the results are representative of the different treatments. One of the limitations of this study was that it did not determine energy expenditure, which could help to explain the weight loss. Another aspect that would have been useful to include was measuring the urinary polyphenols as potential biomarkers of polyphenol intake, as this would help to show the different absorption and excretion rates of cinnamon polyphenols in the participants, which could correlate with the response of the participants to the treatment, and help with assessing compliance. Furthermore, the effects of a high dose of cinnamon in the long term needs to be determined in respect of glucose, CHO and LDL levels.

7.6 Conclusions

Taken together, the findings of the present study demonstrated that consumption of 5 g of *C. cassia* had a beneficial effect on certain markers, in particular a significant decrease in body weight, BMI, waist circumference, blood pressure, and NEFA. In addition, there was a slight reduction in fasting insulin levels, insulin resistance and energy intake. Nevertheless, there was no effect of the cinnamon on fasting glucose, LDL, HDL and total CHO levels. Fasting insulin was positively correlated with NEFA and systolic blood pressure. In addition, insulin resistance correlated with NEFA and systolic blood pressure. Overall, cinnamon supplementation (5 g/d for eight weeks) displayed important health benefits in this overweight group. Cinnamon may be useful as a natural herbal supplement in this setting. Cinnamon might be used as an anti-diabetic and anti-obesity treatment, as well as potentially being used to reduce the risk of diabetes and CVD. The results of this study suggest that future work could further investigate the long term effects of taking cinnamon supplementation by measuring similar parameters, and also focusing on energy expenditure as well as intake, and measuring urinary polyphenols to see if this clarifies the results observed.
CHAPTER EIGHT
Chapter 8

General Discussion
The prevalence of obesity and being overweight is increasing rapidly in many countries around the world, and both conditions are associated with an increased risk of developing type 2 diabetes, hypertension, dyslipidaemia and inflammatory disease (Mokdad et al., 2003; Klop et al., 2013). Losing weight reduces the risk that being overweight has on individuals in terms of developing diseases such as diabetes, and previous studies have shown that changes in lifestyle, such as increasing physical activity, improving diet, reduced-energy intake, pharmacotherapy, and surgery, are each in their own way effective approaches to preventing and treating obesity (Mokdad et al., 2003; Auvichayapat et al., 2008).

Using natural herbal ingredients as a part of the daily diet has attracted a great deal of interest, not only because of their ability in helping to reduce an individual’s weight, but especially because these ingredients do not contain any appreciable energy themselves (Hursel and Westerterp-Plantenga., 2010). Most of the herbal products such as ginger, turmeric, green tea and cinnamon, contain high quantities of several polyphenolic compounds, which can produce beneficial effects within the body (Opara and Chohan., 2014). Westerterp-Plantenga et al. (2006) reported that the consumption of herbal products might produce an effect on metabolic targets such as satiety, thermogenesis, and fat oxidation, which might help in preventing obesity. Cinnamon is an herbal remedy that has been used for over a century; it is the inner bark of the *cinnamomum* tree, which belongs to the Lauraceae family. The main types of cinnamon are *C. zeylanicum* (also known as *C. verum* and *C. cylone*), which is produced in Sri Lanka, and *C. cassia*, which is grown in China (Ravindran et al., 2004; Hamidpour et al., 2015). The hypoglycaemic effects of cinnamon have been established in preliminary studies, both *in vivo* and *in vitro* (Cao et al., 2007). However the effects of consuming *C. cassia* supplements by overweight individuals at an increased risk of developing type 2 diabetes and CVD have not been elucidated. It was hypothesised that daily supplementation with *C. cassia* reduces body weight by regulating glucose utilisation, lowering lipid concentrations, suppressing pro-inflammatory cytokine production, and increasing antioxidant capacity. Therefore,
several methods have been used in this project to investigate the potential health benefits of cinnamon supplement, firstly in vitro and then subsequently in vivo.

Herbal supplements have demonstrated they have potential in disease prevention and health promotion (Opara and Chohan, 2014). In addition, herbal supplements are easily accessible to the general public, compared to prescription pharmaceuticals. Scientific research supports the efficacy and safety of herbal therapies (Cheng et al., 2012), and since 1980, the World Health Organization (WHO) expert committee has encouraged the use of medicinal herbs for treatment and prevention of diseases (Lu et al., 2010).

Cinnamon is one of the most common herbs in the world, it is consumed as a flavouring spice, as cinnamon tea and as an herbal medicine supplement, and it had been used as a traditional medicine for thousands of years (Beejmohun et al., 2014). Many recent studies have reported that cinnamon contains biologically active substances that could be used to treat and prevent some diseases (Cao et al., 2007). This final chapter will review the health properties of cinnamon based on the outcome of a series of studies that have been performed in this project and will link them with current knowledge.

8.1 Anti-oxidant properties of cinnamon supplement samples

Antioxidants are either produced in the body (endogenous) or they are derived from the diet, both of which can directly affect the antioxidant status (Pham-Huy et al., 2008). Cinnamon contains phytochemicals which have powerful antioxidant activities (Dragland et al., 2003). Indeed, cinnamon was ranked as the fourth antioxidant rich food with respect to total antioxidant content, as it contains 17.647 mmol/100 g (Halvorsen et al., 2006). In this clinical long term project that studied the effect of cinnamon, the participants consumed 5 g of cinnamon, which has been shown previously to have a total antioxidant content of 0.882 mmol/g (Halvorsen et al., 2006).

Polyphenols are amongst the natural dietary antioxidants found in cinnamon, which was shown by Helal et al. (2014) who reported that cinnamon contains many phenolic compounds including flavonoids, anthocyanins, and tannins, which increased antioxidant activity and thus offer beneficial health properties. The first objective of this thesis was to determine the polyphenol composition of cinnamon samples and to test their antioxidant activity.
The results (Chapter three) showed that the total phenolic content in *C. cassia* extract was 105.6 mg and in *C. zeylanicum* extract was 101.81 mg of Gallic acid equivalents/g of dry cinnamon. Furthermore, the condensed tannin (proanthocyanidin) contents in *C. cassia* and *C. zeylanicum* were 41.53 mg and 11.42 mg of procyanidin A2/g, respectively. The results of the HPLC and LC-MS analysis indicated that *C. cassia* contained two types of B-type dimers and two types of A-type trimers, while *C. zeylanicum* has only one type of B-type dimers and one A-type trimer. It is clear that the *C. cassia* extract analysed has a higher total phenolic and proanthocyanidin content than *C. zeylanicum* extract. Furthermore, *C. cassia* has a greater variety of types of procyanidin A and B compared with *C. zeylanicum*. A-type procyanidin contains (+) – catechin and/or (-) epicatechin units that doubly link through carbon C2→C7 and C4→C8. B-type procyanidin contains a single link through the C4→C8 bond (Passos et al., 2007). Kondo et al. (2000) reported that procyanidins have antioxidant properties, which is consistent with our findings when the antioxidant activity of the cinnamon extracts were tested. The first antioxidant mechanism that was tested in this project, was the DPPH radical scavenging. The DPPH radical scavenging activity of the *C. cassia* acetone extract was significantly higher when compared with the positive control trolox: 34.99 % and 62.90 % respectively. In addition, *C. cassia* and *C. zeylanicum* acetone (75: 25) extracts possessed a significant (*P* ≤ 0.0001) scavenging activity of H\(_2\)O\(_2\) compared to trolox (0.1 mg/ml): 96.06 %, 92.55 %, and 48.73 %, respectively. Interestingly, the scavenging activity of H\(_2\)O\(_2\) for both *C. cassia* and *C. zeylanicum* extracts were equal to the positive control BHT scavenging activity. However, when looking at the metal chelating activity of the cinnamon extracts, it was found that cinnamon could chelate ferrous ions, but only at a low level when compared to EDTA. This agrees with the results from Mathew and Abraham (2006) who found the metal chelating activity of their cinnamon extract (200 µg/ml) was only 3 %. Although they used different solvent to extract cinnamon which was methanol, they also found that the cinnamon was not as effective at metal chelating as EDTA. In addition, Su et al. (2007) determine the Fe\(^{2+}\) chelating activity of cinnamon that was 0.72 ± 0.08 EDTA equivalents per gram of cinnamon. Also, this study outcome confirmed that cinnamon has a lower Fe\(^{2+}\) chelating activity.

The natural antioxidants such as polyphenols that can be found in the diet can be formulated as functional foods, and may help prevent oxidative damage that occurs
within the body (Hamidpour et al., 2015). The antioxidants identified in cinnamon were polyphenols, such as epicatechin and tannin, and they could potentially replace synthetic antioxidants compounds used in food (Hamidpour et al., 2015). Synthetic antioxidants, such as BHT, have been added to many food products such as oil rich foods and heat-treated foods to protect them from oxidation, and the permitted levels allowed within a food stuff is 100 mg/kg (European Food Safety Authority, 2012). However, this compound is suspected of being carcinogenic and causing liver damage (Barchan et al., 2014), and so there is emphasis within the food industry to reduce its use. Therefore, cinnamon could potentially be used as a safe and natural antioxidant alternative compound in the food industry.

Overall, cinnamon extracts showed an excellent free radical scavenging capacity at all concentrations compared with BHT and trolox, which were used as controls. Cinnamon might donate an electron or hydrogen atom to DPPH radicals and H2O2 to produce stable molecules. The acetone extract of C. cassia was significantly more effective at scavenging free radicals compared with trolox but not-significantly different to BHT. However, cinnamon has a weaker metal chelating activity (8.67 %) compared with EDTA (98.39 %) in this study outcome, which was consistent with the results of the study by Mathew and Abraham (2006). The antioxidant effect of cinnamon might mainly be due to their A-type and B-type procyanidin content, as many studies have reported that procyanidins have antioxidant properties (Kondo et al. 2000; Passos et al., 2007). In addition, in our study the LC-MS analysis of C. cassia confirmed that it contained procyanidins type-B, which agreed with Chen et al. (2012), and Chen et al. (2014), and also procyanidin type-A which agreed with Anderson et al. (2004) who isolated type-A procyanidins from cinnamon and these displayed antioxidant activities.

8.2 Anti-glycaemic properties of cinnamon supplements

Diabetes mellitus (DM) is one of the most prevalent and fastest growing diseases around the world, for example, in Saudi Arabia, Al-Rubeaan et al. (2015) highlighted that more than 50 % of Saudi population is either diabetic or pre-diabetic in a study that was conducted between 2007 and 2009. This is thought to be due to many reasons, such as changes in lifestyle and eating patterns. Recently, there is growing interest in using herbal medicines for the treatment and prevention of DM, and recent in vitro studies have reported that cinnamon contains active substances that mimic
insulin properties, and that it has anti-glycaemic effects (Qin et al., 2003; Khan et al., 2003; Kim et al., 2006). Therefore, the second objective of this series of studies was to test the anti-glycaemic properties of cinnamon supplements both in vitro and in vivo, and in the short and long term, by using a digestive mechanism and in vivo study protocol. In vitro, the effect of cinnamon on the hydrolysis of a starch rich food was determined via RAG and SAG measurements. The method measured the glucose released from a starchy food after the hydrolysis process at 20 and 120 minutes (Englyst et al., 2000). The RAG value of a carbohydrate food correlates strongly with the glycaemic index (GI) value in vivo (Englyst et al., 1996). Englyst et al. (1999) has suggested that RAG might be linked to rapidly digested carbohydrates, which raise blood glucose and insulin response quickly. Conversely, SAG relates to slowly digested carbohydrates, which slow the elevation of blood glucose and the insulin response. The RAG and SAG methodology is based on enzymatic procedures that mimic small intestinal (brush border) digestive enzymes (Englyst et al., 2000; Englyst and Englyst, 2005).

There are a number of factors that have an effect on the glycaemic response in vivo and these can be assessed to some extent in terms of the RAG value in vitro. For example foods rich in phytochemicals might present a low glycaemic response in vivo and a low value of RAG in vitro (Englyst and Englyst, 2005). GI has been shown to be negatively correlated with total phenol content in food in both healthy and diabetic patients (Williamson, 2013). Cinnamon is rich in phytochemicals, including polyphenols, and this study confirmed that both types of cinnamon (C. cassia and C. zeylanicum) had 105.6 mg and 101.81mg of Gallic acid equivalents/g of dry cinnamon, respectively. It is therefore feasible that cinnamon may have an effect on the GI if present in sufficiently high concentrations.

The hypothesis of the study in vitro was that cinnamon polyphenols would interact with the starch hydrolysis process by potentially binding at different sites with digestive enzymes, which could inhibit their function and ultimately the levels of glucose released. This could be used for predicting the effect of the polyphenols on the GI of starchy foods, and it could also help determine the level of cinnamon polyphenols needed to produce an effect in vivo.
The results (Chapter four) showed that the RAG value of cornflakes was reduced in the presence of *C. cassia* and *C. zeylanicum*, the SAG values increased when the cinnamon doses were increased, and low RAG values were associated with high doses of cinnamon, compared with the cornflakes only control. The RAG values in the presence of *C. cassia* and *C. zeylanicum*, were quite similar at all the doses investigated. The addition of 64 mg, 96 mg, 128 mg and 160 mg of *C. cassia* to the cornflakes had a significant reduction on the RAG values (72.1 %, 69.8 %, 68.6 %, 64.4 %, respectively), compared with the controls (81.3 %). Thus, the cinnamon effect is dose dependant; it reduced the RAG value and increased the SAG value. This reduction might be due to the cinnamon polyphenols interacting with the digestive carbohydrate enzymes, α-amylase and α-glycosidase, and inhibiting their function. Ranilla et al. (2010) investigated the effects of *C. zeylanicum* extract on α-amylase activity and they found that cinnamon concentrations of 12.5 and 25 mg/ml inhibited 72 % and 77 % of α-amylase. *C. cassia* in this study was richer in proanthocyanidins (41.5 mg/g equivalent to procyanidin-A2) compared to *C. zeylanicum* (11.4 mg/g equivalent to procyanidin-A2), so this inhibiting effect might be related to cinnamon procyanidin content. A further study that highlighted the ability of procyanidins to inhibit α-amylase was conducted by Gu et al. (2011). They showed that cocoa procyanidins inhibited α-amylase activity by 17-45.5 % at 100 µM.

Acarbose delays the hydrolysis and digestion of complex carbohydrates in the upper small bowel via inhibition of pancreatic α-glucosidase and α-amylase activity, which hydrolyse complex starches to oligosaccharides in the small intestine. Subsequently this slows down the availability of glucose for absorption and helps to control hyperglycaemia after the meal, which means it can be used as a treatment for type 2 diabetes (Standl et al., 2014). As it has a proven effect acarbose was used in this method as a control; the RAG and SAG values for cornflakes in the presence of acarbose were compared with the RAG and SAG values of cinnamon extract. Water-based extracts were prepared for *C. cassia* (*W.E. cassia*), *C. zeylanicum* (*W.E. zeylanicum*) and acarbose (stock concentration, 1 g/ml) at different concentrations (12.8, 25.6, 51.2 µg/ ml). The acarbose can interfere with the freshly prepared enzymes that were added into the cornflakes to digest them. The results showed the effect of acarbose was dose dependent; at the concentrations of 12.8, 25.6 and 51.2 µg/ml decreased the RAG value by 54.77 %, 60.31 % and 66.62 %, respectively. However,
no effect on RAG values was observed for either cinnamon type at any concentration. This lack of effect was probably due to the low concentrations of cinnamon used. At high concentrations of cinnamon, which were added as powder (6.4 mg/ml), a similar effect to acarbose was observed by delaying the release of glucose from starch, possibly by inhibiting the digestive enzymes. The compensation between blank and standards with and without cinnamon demonstrated that cinnamon didn’t interfere with the glucose test. The slow rate of starch digestion could be useful in controlling and lowering the postprandial glucose (Hallfrisch and Behall, 2000). Thus, cinnamon may help to slow the digestion of the cornflakes, and other similar foods, by reducing the RAG value and increasing the SAG value.

The next objective was to determine the acute effect of *C. cassia* supplementation on the glycaemic response in healthy humans. The GI classifies starch rich foods according to their physiological effect on blood glucose (Wolever et al., 1991). The results showed that ingesting 1 g of *C. cassia* supplement 15 minutes prior to consuming 25 g of available carbohydrate (cornflakes) did not change the glycaemic response. In addition, the incremental area under the curve (iAUC) was similar to that demonstrated for the controls. The iAUC results for the glucose drink, cinnamon supplement with cornflakes, and placebo with cornflakes were 145 ± 13, 146 ± 25 and 146 ± 23 respectively. The GI value for the cornflakes when ingested with the cinnamon supplement was 99.0 ± 12, while the GI for the cornflakes only (with placebo) was 98.9 ± 9. This value was similar to the GI value for cornflakes that Wolever and Bolognesi (1996) obtained, which was 99, and shows there was no effect of the treatment.

These results suggest that 1 g of *C. cassia* supplement was not enough to reduce the parameters measured that relate to the blood glucose levels. It is possible there was no effect because the participants were healthy and young; the mean age was 33 years. Indeed, there are some acute studies that support the results of this study, for example Hlebowicz et al., (2009) showed that the addition of 1 g or 3 g of *C. cassia* to 300 g of rice pudding did not have an effect on the blood glucose level of healthy participants. This study with rice pudding fed cinnamon at the same time as the starch rich food which is in contrast to that in the present study where the cinnamon was given before. Nevertheless, no difference was observed.
It is possible that a higher dose of cinnamon, such as 5 g or over, would be required to have an effect on the glycaemic response. However, this would mean that the participants would be consuming about 10 capsules of cinnamon in one go before consuming the cornflakes. This number of capsules is perhaps too high for the participants since it would require quite a long time for them to swallow them, which could also affect the results of the study. In animal studies the cinnamon doses used have been higher, and therefore the effects were more clear, for example, in vivo, oral administration of the cinnamon extract (30 and 300 mg/kg BW) for three weeks enhanced glucose utilization in rats, through stimulating insulin tyrosine phosphorylation of insulin receptor-β and insulin receptor substrate-1 in skeletal muscle (Qin et al., 2003). In our study the daily cinnamon dose was 5 g which was about 65 mg/kg (BW). Therefore, to see an effect in humans by using the same high dose ratio, the dose required about 23 g of cinnamon, which shows that in the acute study the 1 g of cinnamon used was perhaps not enough. Although there was no significant effect of the cinnamon, the outcome of this study helped the investigator to design a study that investigated the effect of consuming cinnamon supplements in the long term.

The long term study investigated the effect of consuming 5 g of *C. cassia* every day for eight weeks on fasting plasma glucose levels, fasting insulin levels and the oral glucose tolerance test (OGTT) results. The results for the fasting plasma glucose levels in the participants who consumed 5 g of cinnamon for eight weeks showed no significant effect on glucose concentrations. However these results are contrary to some other studies, for example many *in vitro* cell culture studies have highlighted that cinnamon improves glucose utilisation and mimics the effects of insulin (Mang et al., 2006). Furthermore, ingesting 1 g, 3 g and 6 g of *C. cassia* daily for 40 days decreased fasting glucose in people with type 2 diabetes (Khan et al., 2003). However our results do agree with a study that found there was no effect of consuming 6 g of *C. zeylanicum* on glucose and insulin response in healthy participants, but this was more to do with the type of cinnamon used not the dose selected (Wickenberg et al., 2012). These results suggest that cinnamon works well with type 2 diabetics, but does not change metabolic parameters in healthy overweight individuals. There may be other reasons for the lack of effect observed in our study; the duration might not have been long enough for the cinnamon to have an effect on the glucose metabolism, as some of the
studies with positive results were longer. Interestingly, the fasting insulin results in the cinnamon group showed there was a trend for reduced insulin concentrations, from 16.43 µU/L at the baseline to 13.05 µU/L at week eight; however, this was not a statistically significant change. The results suggest that cinnamon may reduce the demand for insulin. In addition, insulin resistance in the cinnamon group was reduced from 2.20 at the baseline to 1.82 at week eight, whereas, in control group there was a slight increase. However, again these changes did not reach statistical significance ($P = 0.690$). The lack of significance in these results was because the number in each group was too small, which meant the power of the study was low. The effect at 90% power required 18 individuals for the cinnamon group and 18 for the placebo group, but the number of participants that were involved in the study was small. Anderson et al., (2004) confirms that isolated type-A procyanidin from cinnamon enhances insulin receptors and increases insulin sensitivity in adipocytes *in vitro*. Recently, Kraus et al., (2009) synthesized type-A procyanidins from benzopyrylium salts and catechin, and highlighted the important need for methods to prepare polyphenols derived from cinnamon to allow them to be studied in isolation. Therefore, it might useful to test this synthesized type-A procyanidins *in vitro* and *in vivo*, but the type-A procyanidins may not be absorbed in the gut and so the concentrations tested *in vitro* may not actually be reached in the *in vivo* study, which would need to be taken into consideration when designing the study.

Blevin et al. (2007) found that ingesting 1 g of *C. cassia* every day for three months did not improve fasting glucose, lipid or insulin concentrations in people with type 2 diabetes. It is possible that the dose was too small to see an effect, and the participants were non healthy, as they had type 2 diabetes, which could affect the outcome. In the study by Akilen et al. (2010), 58 poorly controlled type 2 diabetic participants consumed 2 g of *C. cassia* supplement every day for 12 weeks. Their fasting glucose, HbA1c, and systolic and diastolic blood pressures were reduced significantly in the cinnamon group compared with the control group. Moreover, their BMI and waist circumference decreased, although this was not a significant difference. These effects may have been seen because the participants were not able to control their glucose levels, and the number of the participants was large, so this would allow any actual effects to be seen. Ziegenfuss et al. (2006) evaluated the highest cinnamon dose (10 g), which was 500 mg of Cinnulin PF® and equivalent to approximately 10 g of whole cinnamon powder
(i.e. 20:1 extract), which contained at least 1% doubly-linked polyphenol type-A polymers. They investigated the effect of cinnamon on fasting plasma glucose levels among pre-diabetics; who had a baseline fasting plasma glucose of 6.46 mmol/l. After 12 weeks of consuming 10 g equivalent of cinnamon, the fasting glucose and systolic blood pressure were significantly reduced. This effect on fasting glucose and systolic blood pressure might be due to their baseline levels being higher and therefore the effects could be greater in these subjects, as they could reduce to normal levels. Also, the cinnamon supplement was an extract of cinnamon and not cinnamon powder, which could have made it more potent, and the dose was equivalent to 10 g of cinnamon powder, which is high. However, in our study we did not find an effect of using 5 g of cinnamon in the fasting glucose results. This could be explained by the fact that our participants were healthy and their baseline fasting glucose levels were normal and lower than Ziegenfuss et al.'s (2006) participants, and it is harder to lower a parameter that is already in the normal range. Although, consuming 5 g of cinnamon for 8 weeks did slightly reduce insulin levels, there were no changes in the glucose levels. This might be due to the fact that the participants were healthy and their baseline of fasting glucose was within the normal range.

8.3 Anti-inflammatory properties of C. cassia supplements

Inflammation plays a key role in the pathogenesis of a number of chronic diseases such as obesity and CVD (Umar et al., 2015). NSAIDs such as ibuprofen and aspirin, are used to manage inflammatory conditions, however, they have unwanted side effects including intestinal ulcers, bleeding and skin reactions (Rainsford, 1999). These side effects provide the motivation for looking for natural anti-inflammatory treatments with fewer side effects, such as using food and herbal medicines with anti-inflammatory properties (Gunawardena et al., 2015). Cinnamon is a traditional Chinese medicine which was used by the Chinese and Egyptians to treat pain, fever and backache (Lee and Balick, 2005). Some studies report that cinnamon has anti-inflammatory properties in vitro (Gruenwald et al., 2010), therefore the objective of this part of the study was to measure the anti-inflammatory properties of cinnamon in humans. This was done by determining the serum inflammatory marker levels in overweight women after consuming 5g of C. cassia supplement for eight weeks. As far as we know, this study was the first research to investigate the effect of C. cassia supplementation on the extensive profiles of cytokines and adhesion molecules used in overweight healthy...
subjects. Also, the current data regarding the cytokine profiles in healthy overweight and older individuals has been limited, therefore, the data produced in this study might be used to apply for further research to study the cytokine profiles in these groups.

The inflammatory markers measured in this study were IL-6, IL-8, IL-10, MCP-1, TNF-α, CRP, VCAM, ICAM, E-selectin, P-selectin and L-selectin. The results show that all of these markers, before and after treatment, were within the normal range.

The findings from the data showed that IL-6 levels were significantly increased due to the duration effect ($P < 0.05$), and the baseline level of IL-6 in the cinnamon group was in the low normal range. These results may be due to the fact that IL-6 has both anti-inflammatory and pro-inflammatory functions. Pini et al. (2013) and Wallenius et al. (2002) both stated that obesity is associated with high levels of IL-6 and also IL-6 deficiency. The cinnamon group saw a significant reduction in their weight and this may have helped to change the levels of IL-6. Elevated IL-6 concentrations are also associated with insulin resistance and high systolic and diastolic blood pressure (Bermudez, et al., 2002). Although we found that the IL-6 concentration increased in the cinnamon group, the insulin resistance reduced slightly and blood pressure was reduced significantly by the treatment.

The increased levels of IL-6 levels were still in the normal range, so there should be no specific concern as to the effects of cinnamon on this marker. Nevertheless, acute IL-6 treatment has been shown to increase insulin stimulated glucose disposal (uptake), and increase fatty acid oxidation in vitro (Carey et al., 2006). Although the significant increase in IL-6 concentration was due to the effect of duration, the mean IL-6 concentration in the cinnamon group at weeks four and eight increased, whereas the mean IL-6 concentration in the control group did not change. Therefore this suggests the increasing IL-6 level was not a risk marker, as this is in the normal range. It might be that increasing the IL-6 in the maximum normal range is a good target to have, as it may have health benefits such as a reduction in weight.

The variability in IL-6 over time might suggests there was another reason for the increased levels of IL-6. In Biancottoet et al. (2013) study 27 cytokines levels were determine in 144 healthy participants, age between 21-62 year, at baseline and after 7 days. Their outcome showed a higher variability between the cytokines levels over time. Furthermore, the circulation of TNF-α and IL-6 levels were determined at the
baseline (day 1), 2, 3, 8, 15, 22, 50 and day 78 in 10 patients with heart failure and 10 healthy subjects. The variation for TNF-α and IL-6 levels increased over time in both groups (healthy and heart failure) and there were no significant differences between heart failure and control subjects (Dibbs et al., 1999). Thus, the variability is expected in the cytokines levels over time in healthy and heart failure patients and this suggests any small effect caused by cinnamon would be masked by the natural change.

Several studies have shown that IL-6, TNF-α and CRP increase with age in healthy and in overweight or obese individuals (Singh and Newman, 2011). This was not noticeable in our study participants either at the baseline or during the treatment, even though they were older, aged over 53, and healthy overweight. There was also no significant effect of the cinnamon supplementation on TNF-α and CRP levels, but there was a slight reduction in CRP and TNF-α in the cinnamon group, while these did not change in the control group. Moreover, the slight reduction in CRP and TNF-α in the cinnamon group was associated with a significant reduction in weight. There was evidence to suggest that the weight lowering property of fruit and green teas in overweight populations is related to their polyphenols, as these might alter the gut microflora. In vitro, the metabolism of proanthocyanidins via microflora can derive anti-inflammatory properties, which reduce the secretion of TNF-α, IL-1b and IL-6, so it is possible that this effect is occurring in vivo in the participants in this study (Cardona et al., 2013).

One of the other markers we investigated was IL-10, which is a cytokine that suppresses the immune response and inhibits the production of pro-inflammatory and systemic inflammatory compounds such as TNF-α and CRP (Lee et al., 2002). Interestingly, the anti-inflammatory IL-10 concentration increased slightly during the treatment from 0.88 pg/ml at the baseline to 1.07 pg/ml at week eight in the cinnamon group; but this was not significant. However, in the control group the IL-10 levels were almost the same throughout the experiment. It seems that cinnamon, in addition to the effect of time, increased the cytokine’s network and lead to an efficient inflammatory response, which can confer high resistance to infectious diseases.

Animal studies have confirmed that cinnamon has an effect on inflammatory markers. The study showed that feeding an aqueous cinnamon extract orally at doses of 20 mg/kg and 100 mg/kg to mice, which have high serum IL-6 and TNF-α levels, for six
days reduced both IL-6 and TNF-α levels significantly (Hong et al., 2012). In a recent in vitro study, Gunawardena et al. (2015) emphasised that E-cinnamaldehyde in cinnamon extracts has the ability to inhibit TNF-α production in RAW 264.7 macrophages. This research suggests the cinnamon polyphenols content, such as type A and B procyanidins and catechin, and E-cinnamaldehyde, may be the main active biological compounds in cinnamon which gives it the anti-inflammation properties.

The investigation of the adhesion molecules in this study showed there was no significant effect of cinnamon supplementation on the levels of the markers, which included sVCAM, sICAM, sE-selectin, sP-selectin and sL-selectin. Interestingly, there was a slight reduction in the levels of sVCAM, sICAM, sE-selectin, sP-selectin and sL-selectin, which would suggest the cinnamon was having an effect, but the number of participants was too low for this to be significant. Adhesion molecules including sVCAM, sICAM, sESEL, sPSEL and sLSEL are on the cell surface of vascular endothelial cells and are involved in endothelial function (Carlos and Harlan, 1994). It seems that cinnamon improves the endothelial function and reduces the sVCAM, sICAM, sESEL, sPSEL and sLSEL concentrations slightly. There have been no studies in humans using cinnamon supplementation that have investigated the effects on adhesion molecule markers to compare with our outcomes. However, there were some studies that tested the effect of food polyphenols on some adhesion molecule markers. Barona et al. (2012) studied the effect of consuming 46 g/d of grape polyphenol supplements for 30 days on 25 participants with metabolic syndrome, who were at high risk of developing high blood pressure. The results of this crossover study showed a significant decline in sICAM-1 concentrations by 10 µg/L (142 ± 50 µg/L), in the grape group compared with placebo (151 ± 51 µg/L), whereas, the plasma sVCAM-1 levels did not differ between the grape (1020 ± 285 µg/L) and placebo (1020 ± 240 µg/L). In our study, there were no significant effects on the adhesion molecule markers. This is possibly related to the small number of the participants that were involved in this study.

8.4 Lipid lowering properties

According to some previous studies, cinnamon has a positive effect on lipid profiles (Khan et al., 2003). Therefore, an objective of this study was to determine the effect of ingesting 5 g of C. cassia supplementation on lipid profiles which included CHO, HDL,
LDL, TAG and NEFA. Previous studies have examined some of these markers, but according to our knowledge this study was the first to look at the effect of cinnamon on NEFA levels. The findings (Chapter seven) highlighted that NEFA concentrations decreased significantly after ingesting 5 g of *C. cassia* (*P* = 0.017). Elevated plasma NEFA concentrations are associated with insulin resistance (Karpe et al., 2011). As mentioned above, insulin resistance was reduced slightly in the participants consuming cinnamon, which might be as a result of the NEFA concentrations being reduced significantly. This finding might go some way to reduce the risk of type 2 diabetes and CVD, as Frayn and Williams (1996) stated that elevated plasma NEFA concentrations might be a marker for metabolic syndrome, type 2 diabetes and CVD. Our data demonstrated that reducing NEFA levels was correlated with a reduced fasting insulin level and insulin resistance (*P* = 0.030, *R* = 0.560; *P* = 0.028, *R* = 0.566, respectively).

Within the literature there was no studies on cinnamon and the effect on the NEFA concentration to compare our results with. Within our results there was no significant change in CHO, HDL, and LDL levels after consuming 5 g of *C. cassia*. These findings agree with Mang et al. (2006) who found that ingesting 3 g of cinnamon capsules for four months did not change CHO, HDL, and LDL levels in 79 type 2 diabetes participants. Also in another study, ingestion of 1.5 g of *C. cassia* supplements for six weeks did not change CHO, HDL, and LDL concentrations in cinnamon group (placebo: 13, cinnamon: 12) (Vanschoonbeek et al., 2006). Overall from these results it is clear that cinnamon did not change the CHO, HDL, and LDL concentrations, though did significantly reduce NEFA levels.

### 8.5 Blood pressure lowering properties

Hypertension is one of the major risk factors for developing CVD, therefore focus on the management of blood pressure has shifted from conventional pharmacologic approaches, as the therapeutic powers of plants have become more widely recognised (Perez-Vizcaino et al., 2009). Plant, fruit and herbs might contain specific components that reduce blood pressure, such as polyphenols (Perez-Vizcaino et al., 2009). Cinnamon is one of the promising herbal supplements that could be used to help reduce blood pressure, but data on its blood pressure lowering potential is limited. Therefore, an objective in this study was to look at the effect of 5 g of *C. cassia* on resting blood pressure.
The outcome of this cinnamon study on resting blood pressure showed that 5 g per day of *C. cassia* supplementation for eight weeks reduced blood pressure significantly; the systolic blood pressure was reduced at both weeks four and eight to 114.75 mm Hg and 105.43 mm Hg, respectively (*P* = 0.04 and *P* = 0.01 respectively). The diastolic blood pressure decreased significantly at week eight to 73.28 mm Hg compared with the baseline 83.00 ± 3.2 (*P* = 0.02), while there were no changes observed in the control group for either blood pressure measure. According to a recent meta-analysis that was run by Akilen et al. (2013), there were only three randomised controlled trials that studied the effect of cinnamon on blood pressure. In the Akilen et al. (2010) study, the cinnamon (*C. cassia*) dose was 2 g/d for 12 weeks, (N = 58). This cinnamon daily dose significantly reduced the systolic and diastolic blood pressure (*P* < 0.001). The baseline of the systolic and diastolic blood pressure in the cinnamon group was 133 mm Hg and 85 mm Hg respectively, while the placebo group was 135 mm Hg and 86 mm Hg, respectively. After the treatment, the systolic and diastolic blood pressure of the cinnamon group was 129 mm Hg and 81 mm Hg respectively, while the placebo group was 134 mm Hg and 87 mm Hg, respectively, which is similar to the results of our study. Another study found that ingesting 500 mg of daily cinnamon extract (Cinnulin PF®), which is equivalent to 10 g of cinnamon, for 12 weeks reduced systolic blood pressure significantly within the cinnamon group (*n* = 12) from 133 ± 14 mm Hg [pre] to 128 ± 18 mm Hg compared with the placebo group (*n* = 10) (Ziegenfuss et al., 2006). On the other hand, Wainstein et al. (2011) found a marginal effect (*P* = 0.06) of 1.2 g/d (*n* = 59) of cinnamon for 12 weeks on blood pressure, which might be because they only used a low dose of cinnamon. Overall these results suggest that the effect of cinnamon on blood pressure is dose dependent. Also, in the previous study, the baseline of blood pressure was high, and the higher the blood pressure the greater the chance it has to decrease, so the participants demonstrated a reduction in their blood pressure. However, in our study the participant’s blood pressure was within the normal range at baseline.

Preuss et al. (2006) reported that nutrients and drugs that enhance insulin sensitivity or reduce insulin levels, also have the capacity to lower blood pressure in rats consuming high levels of sugars. This fits with our data as there was a significant reduction in blood pressure associated with a slight decrease in insulin resistance (Chapter six). This means that cinnamon components significantly reduced systolic
and diastolic blood pressure, which led to slight reduction in fasting insulin. Our outcome showed that systolic blood pressure positively correlated with fasting insulin and insulin resistance \((P = 0.030, R = 0.561; P = 0.024, R = 0.579, \text{respectively})\).

Damaged endothelial cells in blood vessels express high concentrations of adhesion molecules, which increases the inflammation and might lead to an increased blood pressure (Borana et al., 2012). CRP has been found to be strongly associated with an increased risk of hypertension in women (Sesso et al., 2007). There is also evidence that blood pressure is related to adhesion molecules, such as ICAM, VCAM, sESEL, sPSEL and sLSEL and CRP, and thus their concentrations could be used as a risk marker of hypertension and CVD. This evidence was confirmed by the Ghanem et al. (2007) study which showed that sPSEL and sESEL were positively correlated with hypertension \((R = 0.512, P = 0.051; R = 0.523, P = 0.045, \text{respectively})\). Our data agree with this correlation that sPSEL correlated with systolic blood pressure \((R = 0.512, P = 0.051)\), and in addition there was a positive correlation between sESEL and systolic blood pressure \((R = 0.523, P = 0.045)\). For this reason, one of our objectives (Chapter six) was to determine the inflammatory marker levels, and as previously stated our findings showed a small (but not significant) reduction in adhesion molecule markers ICAM, VCAM, sESEL, sPSEL and sLSEL and CRP, which might be related to the lowered blood pressure observed.

### 8.6 Weight reducing properties

The prevalence of obesity and being overweight is increasing worldwide, and obesity increases the risk of chronic diseases such as diabetes mellitus and CVD, including hypertension (Auvichayapat et al., 2008). The main cause of obesity and being overweight is an imbalance between energy intake and energy expenditure. There are many strategies to reduce weight, such as reducing energy intake, increasing exercise levels, and increasing the consumption of food rich in polyphenols which leads to greater satiety, energy expenditure and fat oxidation (Westerterp-Plantenga et al., 2006; Rastmanesh et al., 2011).

The findings from assessing body weight during the eight weeks showed that the group taking the *C. cassia* supplement lost about 2 kg, while no changes were recorded in the placebo group after both four and eight weeks. By analysing the food intake data of the cinnamon group, the results over the first four weeks showed a slight reduction
in the total energy intake (from 1753.13 kcal to 1708.37 kcal), which could be the reason why 1 kg was lost after four weeks of consuming cinnamon supplementation. By the end of the experiment, the participants had lost about 2 kg. In addition to the energy reduction, it might be that cinnamon components effect satiety and delay the gastric emptying rate; however, this study did not measure either of these parameters to confirm this. A further explanation for the weight loss was that the fasting insulin data showed a marginal decrease from 16.43 µU/l at the baseline to 13.05 µU/l at week eight; however, this was not a statistically significant difference. In addition, insulin resistance in the cinnamon group was reduced slightly from 2.20 at the baseline to 1.82 at week eight, whereas in the control group there was a slight increase. Also the lipid profile outcomes showed that NEFA was reduced significantly after consuming 5 g of cinnamon ($P = 0.017$). This might be confirmation that cinnamon has a positive effect on fat metabolism, which leads to a reduction in weight. Other studies have shown that consuming 525 mg of green tea extract in capsular form, which contained catechin polyphenol and caffeine, for 6 weeks significantly increased energy expenditure (4 %), and carbohydrate and fat oxidation in a 24-hour period, but did not increase the heart rate (Dulloo, 1999). In another green tea study, sixty participants (green tea: 30, placebo: 30) consumed 750 mg of green tea or placebo in capsules form every day for 12 weeks. The participants’ weight in the green tea group decreased significantly, and the total reduction was 3 kg during 12 weeks (Auvichayapat et al., 2008). It seems that green tea increased energy expenditure, which agrees with our results that polyphenol containing plants and herbs can influence weight loss.

### 8.7 Concluding remarks

This thesis has examined the health properties of cinnamon supplementation through different approaches both *in vitro* and *in vivo*. The *in vitro* results have shown that cinnamon has active components and performs well in terms of antioxidant activities. Furthermore, the components of cinnamon might inhibit digestive enzymes and reduce their function, which suggests this could thus be used as an alternative to acarbose that is used as a treatment for type 2 diabetes. The *in vitro* data showed that the effect of cinnamon was dose dependent as shown by the results for the antioxidant activity approaches, and the RAG and SAG values. In the human study, *C. cassia* supplementation (5 g/d for eight weeks) displayed important health benefits in overweight individuals that were at risk of developing type 2 diabetes and CVD.
results showed a significant effect in NEFA, weight, blood pressure and IL-6 levels, and a slight decrease in some inflammatory markers such as CRP, ICAM and VCAM. Overall this study suggests cinnamon may be useful as a natural herbal supplement for overweight people trying to control their weight, and cinnamon has the potential be used as an anti-diabetic, anti-inflammatory, anti-obesity product and could reduce the risk of CVD by reducing the blood pressure.

8.8 Future work

- Further studies are needed to confirm the health impact of consuming *C. cassia* in type 2 diabetic and hypertensive participants.
- Comparison of the GI of different rich starchy foods that are consumed with cinnamon (*C. cassia* and *C. zeylanicum*).
- Comparison of the GI of rich starchy foods that are consumed with *C. cassia* and at high dose, five grams and over.
- Comparison of the GI of rich starchy foods that are consumed with *C. zeylanicum* and at high dose, five grams and over.
- Investigate the effect of cinnamon on the digestive enzyme α-amylase in pure form.
- Determine the effect of cinnamon on energy expenditure.

All of these studies can follow a similar protocol as described in this thesis, but to make the results statistically significant they need to recruit a larger number of participants for the treatment and control groups.
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References


References


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References


References


References


Appendices

Appendix 1

Trabecular density

Scan site of bone

peripheral Quantitative Computed Tomography (pQCT)
Appendices

Appendix 2

Capsule filling machine
Appendices

Appendix 3

Evidence Investigator

Biochips

Labelled antibody (conjugate)

Analyte in patient sample

Antibody bound to biochip surface

Biochip

Sandwich biochip principle
Appendices

Appendix 4
Ethic letter Approval Ethics Committee (EC/2011/145/FHMS)

Dr Jonathan Brown
Dept of Nutrition and Metabolism
FHMS

09 August 2012

Dear Dr Brown

Assessment of the effect of a cinnamon supplement on the glycaemic index and insulin response of a starch rich food EC/2011/145/FHMS

I am writing to inform you that the Chairman, on behalf of the Ethics Committee, has considered the Amendments requested to the above protocol and has approved them on the understanding that the Ethical Guidelines for Teaching and Research are observed. Please be advised that the Ethics Committee is able to audit research to ensure that researchers are abiding by the University requirements and guidelines.

If the project includes distribution of a survey or questionnaire to members of the University community, researchers are asked to include a statement advising that the project has been reviewed by the University's Ethics Committee.

Date of confirmation of ethical opinion: 20 March 2012.

Date of favourable ethical opinion of amendment to protocol: 9 August 2012.

The list of amended documents reviewed and approved by the Chairman is as follows:

<table>
<thead>
<tr>
<th>Document</th>
</tr>
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<tbody>
<tr>
<td>Amendment of title</td>
</tr>
<tr>
<td>Protocol Cover Sheet</td>
</tr>
<tr>
<td>Summary of the Project</td>
</tr>
<tr>
<td>Detailed Protocol</td>
</tr>
<tr>
<td>Screening Questionnaire</td>
</tr>
<tr>
<td>Participant Information Sheet</td>
</tr>
<tr>
<td>Consent Form</td>
</tr>
<tr>
<td>Risk Assessment</td>
</tr>
<tr>
<td>Recruitment advert</td>
</tr>
</tbody>
</table>

Yours sincerely

Glenn Moulton
Secretary, University Ethics Committee
Academic Registry
Appendix 5

Questionnaire

Date .............................................  Reference number: ............

PERSONAL DETAILS

Title: .............................................  DOB: ...../...../......

Family name: .................................  Age: .................

Initials: .........................  (18 – 60)

Address: .................................

.............................................

Telephone:  Day .................

Evening.................

Weight (kg): ..........  Height (m): ...........

GENERAL HEALTH

<table>
<thead>
<tr>
<th>Condition/Disorder</th>
<th>YES</th>
<th>NO</th>
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</thead>
<tbody>
<tr>
<td>Psychiatric Disorders</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drug/Alcohol dependence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epilepsy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sleep Disorders e.g. Insomnia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endocrine Disorders (inc. Diabetes)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiovascular Disease</td>
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<td></td>
</tr>
<tr>
<td>Blood Pressure-Hypertension</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renal Disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatic Disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastrointestinal Disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cinnamon allergic</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Any food allergies  YES/NO

IF YES:

Food/s  ........................................................................................................

GENERAL QUESTIONS

Nicotine exposure (please tick the relevant box)  Smoker  □ How many a day...........

Non-smoker  □

Do you consume Indian food?  Yes  □  No  □
Do you drink cinnamon tea? Yes ☐ No ☐
Have you ever added cinnamon powder to your drinks? Yes ☐ No ☐
Have you ever suffered ill effects after consuming cinnamon rich foods? Yes ☐ No ☐

Average caffeine consumption/day
How many cups/day:-
Tea…………………………
Decaffeinated tea………..
Herbal tea………………..
Coffee……………………
Decaffeinated coffee……
Cola ........................

Average alcohol consumption/week ........................

Pint of beer is 2 units, 125 ml Glass of wine is 1 unit
Appendices

Appendix 6

PARTICIPANT INFORMATION SHEET:

PROJECT TITLE: Assessment of the effect of a cinnamon supplement on the glycaemic index and insulin index of a starch rich food

INSTITUTION: Department of Nutrition and Metabolism, Faculty of Health and Medical Sciences, University of Surrey

INVESTIGATORS: Tahany Aldayel, Dr Jonathan Brown, Professor Susan Lanham-New

A: INTRODUCTION

We would like to invite you to take part in a research project. Before you decide you need to understand why the research is being done and what it will involve for you. Please take the time to read the following information carefully. Talk to others about the study if you wish.

B: BACKGROUND

Many herbal products are now available that claim to have health beneficial properties. Cinnamon is an example of this which has been claimed to help lower high blood sugar levels. Furthermore cinnamon is a traditional remedy used in some countries in the treatment of diabetes. Cinnamon contains some important components that may help the body use sugar more appropriately. Sugar in the body comes from foods that contain carbohydrates and the ability of the body to use this sugar has an effect of the amount of sugar in the blood. The glycaemic index approach is a method used to rank carbohydrate rich foods based on their ability to affect blood glucose (blood sugar) levels. This project will involve an investigation into the effect of a cinnamon supplement on the glycaemic index (GI) and insulin response of a starch rich food and to compare it with the glycaemic index of the same food without the cinnamon supplement. The amount of carbohydrate in these foods is known and the responses produced will be compared to the same amount of carbohydrate in a reference food (glucose).

We will ask you to attend seven study legs in total in order to complete the study. The study will take place at the Clinical Investigation Unit (17AX00) of the Faculty of Health and Medical Sciences. Each leg will last a maximum of 3 hours and be carried out in the morning between 08.00 hours and 11:00 hours. On two occasions you will consume two capsules (total 1000 mg) of the cinnamon supplement (Holland and Barrett) 15 minutes prior to the cornflakes; on two further days you will consume two placebo tablets 15 minutes prior to the cornflakes. On the three other study legs/occasions you will consume the reference food (glucose drink). There will be at least two days between study days.

This study uses a WHO/FAO protocol for assessing the glycaemic index (GI) of the starch rich food. The aim of the study is to see whether consuming the cinnamon supplement before the starch rich food has an effect on the GI and insulin response.

YOU CAN ONLY TAKE PART IN THIS STUDY IF YOU ARE:

1. Aged 18-60 years
2. Free from medication with the exception of minor analgesics e.g. paracetamol
3. Free from cinnamon allergy
YOU CANNOT TAKE PART IN THIS STUDY IF YOU:

1. Have a familial or personal history of psychiatric disorders, epilepsy, sleep disorders, diabetes, CHD or hypertension, liver disorder or food allergies
2. Have suffered from infectious hepatitis, jaundice, malaria, tuberculosis and are not HIV positive
3. Have a history of drug or alcohol abuse
4. Have taken any medication (with the exception of minor analgesics or oral contraceptives) within 2 weeks of the start of the study
5. Have received an experimental drug either clinically or recreationally within the past 3 months
6. Have a phobia of needles/sight of blood
7. Are or may be pregnant
8. Have cinnamon allergy

C: PRELIMINARY

1. SCREENING

As part of the screening process, you will be asked to answer a screening questionnaire to determine your general health status (Appendix 1) and to assess your suitability to participate in the study. Depending on the results of your screening evaluation, you may then be eligible to participate. All information obtained will be restricted to the investigators only and will be kept strictly confidential. All participates must sign a consent form.

2. PROCEDURES TO BE FOLLOWED

For 24 hours prior to the study day, you must refrain from consuming alcohol and caffeine-containing drinks (tea/coffee/carbonated soft drinks such as Pepsi, Coco-cola, Red Bull) and avoid strenuous exercise throughout the day before and during the study day. You will not be allowed to consume any food or drink, with the exception of water, from 20:00 h prior to the study day.

3. EXPECTED DURATION OF THE STUDY

You will be asked to attend the Clinical Investigation Unit on seven occasions during the day (08:00 hours-11:00 hours). There will be at least two days between each study day.

4. NUMBER OF SUBJECTS EXPECTED TO PARTICIPATE

You will be one of approximately 10 subjects who will participate in this study

D: Protocol

Study day 1

You will be required to arrive at the Clinical Investigation Unit (Room 17 AX 00) at 08:00h on the day of the study. Blood samples will be collected by finger pricks using preset lancets 8 samples for each study leg with cinnamon supplement and placebo, whereas reference legs required 7 samples. One basal sample will be collected at the start of the study (-15 minutes). You will then be asked to consume two capsules (Cinnamon or placebo) +100 ml of water. After 15 minutes (0 minutes) a further blood samples will be taken after that you will be asked to eat a portion of cornflakes (30 g). Finger prick blood samples (No less than 300 µl per sample) will be collected at -15, 0, 15, 30, 45, 60, 90 and 120 minutes. Thus, the total blood sample is 8 samples. On the three other study legs/occasions you will consume the reference
food (glucose drink). One basal sample will be collected at the start of the study (time 0 min). Then, finger prick blood samples (No less than 300 μl per sample) will be collected at 15, 30, 45, 60, 90 and 120 minutes after have the reference which is glucose drink. Thus, the total blood sample in reference legs is 7 samples. The total volume of blood taken will be less than 3 ml allowing for wastage.

You will be required to remain seated throughout the study, except for visits to the toilet.

A member of the research staff will be present throughout the study.

Study days 2, 3, 4, 5, 6 and 7

These will be identical to study leg 1. The order in which the capsules (placebo or cinnamon) are given will be randomized.

**E: GENERAL**

All information and data obtained from this study will be restricted to the investigators only and will be kept strictly confidential. The data obtained from all participants will be used to establish the effect cinnamon on the glycaemic index and insulin response, and once the study is complete a summary of the work will available to each participant. It is hoped that the work will also be published at a conference. You will be free to withdraw from the study at any time, without needing to justify your decision and without prejudice.

**F: ANALYSIS**

Blood samples collected during the study will be analyses for glucose and insulin. All samples will be analyses at the Faculty of Health and Medical Sciences, University of Surrey. After which they will be disposed of in accordance with the University rules of disposal of biological materials.

**G: PAYMENT FOR PARTICIPATION**

A sum of £20 per study occasion (i.e. each study leg) will be paid to you on completion of the study as out of pocket expenses if you complete the 7 study occasions. You will be paid less if you withdraw from the study early but this is at the discretion of the Principal Investigator.

**H: DISCOMFORT ASSOCIATED WITH STUDY**

There is a possibility that you may experience slight discomfort during finger prick sampling. You may have sore or bruised fingers due to multiple finger pricks initially when trying the finger prick technique. However previous experience over the last 5 years has shown this to be very unlikely. If this was to be the case then you could increase the number of rest days or withdraw from the study. The equipment used in this procedure is similar to that used for monitoring blood glucose by diabetics.
I: CONTACT DETAILS

If you should have any questions regarding any aspect of this study, please contact:

Tahany Aldayel
Faculty of Health and Medical Sciences
University of Surrey
Guildford
GU2 7XH
E-mail: T.Aldayel@surrey.ac.uk

Or Dr Jonathan Brown
Faculty of Health and Medical Sciences
University of Surrey
Guildford
GU2 7XH
E-mail: J.E.Brown@surrey.ac.uk

Thank you for taking time to read this Information Sheet
Appendices

Appendix 7
Consent Form:

- I the undersigned voluntarily agree to take part in the study on Assessment of the effect of cinnamon supplement on the glycaemic index and insulin response of a starch rich food.

- I have read and understood the Information Sheet provided. I have been given a full explanation by the investigators of the nature, purpose, location and likely duration of the study, and of what I will be expected to do. I have been advised about any discomfort and possible ill-effects on my health and well-being which may result. I have been given the opportunity to ask questions on all aspects of the study and have understood the advice and information given as a result.

- I agree to comply with any instruction given to me during the study and to co-operate fully with the investigators. I shall inform them immediately if I suffer any deterioration of any kind in my health or well-being, or experience any unexpected or unusual symptoms.

- I consent to my personal data, as outlined in the accompanying information sheet, being used for this study and other research. I understand that all personal data relating to volunteers is held and processed in the strictest confidence, and in accordance with the Data Protection Act (1998).

- I understand that I am free to withdraw from the study at any time without needing to justify my decision and without prejudice.

- I acknowledge that in consideration for completing the study I shall receive the sum of £20 per leg. I recognise that the sum would be less, and at the discretion of the Principal Investigator, if I withdraw before completion of the study.

- I understand that in the event of my suffering a significant and enduring injury (including illness or disease) as a direct result of my participation in the study, compensation will be paid to me by the University (or sponsor where a clinical trial is sponsored by a pharmaceutical company), subject to certain provisos and limitations. The amount of compensation will be appropriate to the nature, severity and persistence of the injury and will, in general terms, be consistent with the amount of damages commonly awarded for similar injury by an English court in cases where the liability has been admitted.

- I confirm that I have read and understood the above and freely consent to participating in this study. I have been given adequate time to consider my participation and agree to comply with the instructions and restrictions of the study.
Appendices

Name of volunteer (BLOCK CAPITALS)
...........................................................................

Signed ........................................................................

Date ............................................................................

Name of witness (where appropriate) (BLOCK CAPITALS)
.............................................................................

Signed ........................................................................

Date ............................................................................

Name of researcher/person taking consent (BLOCK CAPITALS)
...........................................................................

Signed ........................................................................

Date ............................................................................
Appendices

Appendix 8

Dr Jonathan Brown
School of Biosciences & Medicine
FHMS

19 December 2013

Dear Dr Brown

An assessment of the effect of a daily cinnamon supplement on inflammatory markers, lipid profile, glucose tolerance and insulin response EC/2013/70/FHMS

On behalf of the Ethics Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the submitted protocol and supporting documentation.

Date of confirmation of ethical opinion: 19 December 2013

The final list of documents reviewed by the Committee is as follows:

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<tr>
<td>Summary of the project revised version 19 November 2013</td>
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<td>Revised Detailed protocol version 18 December 2013</td>
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<td>Cover Letter dated 18 December 2013 with response regarding recruitment of individuals.</td>
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<tr>
<td>Participant Information Sheet</td>
</tr>
<tr>
<td>Consent Form</td>
</tr>
<tr>
<td>Study Screening Sheet</td>
</tr>
<tr>
<td>Recruitment advert</td>
</tr>
<tr>
<td>Risk assessment</td>
</tr>
</tbody>
</table>

This opinion is given on the understanding that you will comply with the University's Ethical Principles & Procedures for Teaching and Research.

If the project includes distribution of a survey or questionnaire to members of the University community, researchers are asked to include a statement advising that the project has been reviewed by the University’s Ethics Committee.

If you wish to make any amendments to your protocol please address your request to the Secretary of the Ethics Committee and attach any revised documentation.

The Committee will need to be notified of adverse reactions suffered by research participants, and if the study is terminated earlier than expected with reasons. Please be advised that the Ethics Committee is able to audit research to ensure that researchers are abiding by the University requirements and guidelines.

You are asked to note that a further submission to the Ethics Committee will be required in the event that the study is not completed within five years of the above date.

Please inform me when the research has been completed.

Yours sincerely

Mike Chenery
Secretary, University Ethics Committee
Research & Enterprise Support
Appendix 9

“Would you like to take part in a cinnamon study”

“We are looking at the health benefit of consuming cinnamon supplement in term of inflammatory markers, lipid profiles, glucose tolerance and insulin response”

We are looking for:
Healthy Women
Aged 45-70
With a BMI >25

If you’re interested please contact Tahany Aldayel e-mail T.Aldayel@surrey.ac.uk, mobile number 07450095509 Or telephone (01483682599).

This study has received a favourable ethical opinion from the University of Surrey Ethics Committee.
Appendices

Appendix 10

Study screening sheet

Assessment of the effect of a daily cinnamon supplement on the inflammatory markers, lipid profiles, glucose tolerance and insulin response

Participant screening sheet

Date .................. Reference number:......................

PERSONAL INFORMATION

Name: .................................................................
Age: .................................................................
Date of birth: ...........................................................
Address: ........................................................................
...........................................................................
...........................................................................
...........................................................................

Contact telephone numbers:
........................................................................
........................................................................

Email address: ...........................................................

Contact details of someone who could be contacted in an emergency:
........................................................................
........................................................................

Weight (kg): .................... Height: ................. BMI: .............

Blood pressure: R1 .............. R2 .............. R3 ............. Average: ..............

Heart rate:.............. .............. .............. Average: ..............
Appendices

Fasting Glucose level (finger prick): …………………….

Waist circumference: …………………………              Hip circumference ………………………

Please tick all/any of the following that apply:

☐ I am free from any health and medical problems
☐ I am not currently taking or have taken any supplements
☐ I am not currently taking or have taken any herb or plant supplements
☐ I am not currently taking or have taken any steroid drugs
☐ I have no prior/present history of kidney disease
☐ I have no prior/present history of kidney stone disease
☐ I have no prior/present of fracture incidence
☐ I have no prior/present history of Epilepsy
☐ I have no prior/present history of Cinnamon allergy
☐ I have no prior/present history of Coronary Heart Disease, Angina or Stroke
☐ I have no prior/present history of Type 1 or 2 diabetes
☐ I have no prior/present history of anaemia
☐ I have no prior/present history of hormone disorders
☐ I have no prior/present history of gastrointestinal diseases (for example Crohn’s disease, Coeliac disease, Irritable Bowel Syndrome)
☐ I have no prior/present history of liver disease
☐ I have no prior/present history of being treated for depression and/or other psychological disorders
☐ I have no prior/present history of eating disorders, including anorexia or bulimia nervosa
☐ I have no prior/present history of drug or alcohol abuse within the last 2 years
Are you suffering from frequently of infection? YES/ NO
If yes, how often and what do you take.

Are you currently on a weight-reducing diet or other dietary restriction? YES/ NO
If yes, please provide details.

Are you taking Non-steroidal anti-inflammatory drugs (NSAIDs) such as Ibuprofen, Aspirin, Voltaren, Lodine and Anaprox? YES/ NO
If yes, please provide details.

Are you allergic to any foods? YES/ NO
If yes, please state what foods you are allergic to.

Have you ever suffered ill effects after consuming cinnamon rich foods? YES/ NO
If yes, please provide details.

Have you been involved in a clinical trial in the last 3 months? YES/ NO

Have you taking any regular medication prescribed by my GP in the last 6 months YES/ NO
If yes, please state what you are taking.

Have you taking painkiller regularly such as Paracetamol, Panadol and Anadin YES/ NO
If yes, how often and at what the dose?

Do you exercise regularly? YES/ NO
If yes, what type of exercise and how often?

Do you take any dietary supplements such as vitamins, minerals or fish oils? YES/ NO
If yes, please state which type and how often.

Do you consume a caffeine drink such as tea, coffee or cola and power drink? YES/ NO
If yes, how many cops per day (give details please)
Appendices

Do you smoke? YES/ NO
If yes, how many cigarettes per day?

Do your menstrual cycles come regularly every month? YES/ NO
If no, how often it is come?

Do you drink alcohol? YES/ NO
If yes, how many units per week? (See below)

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<thead>
<tr>
<th>Alcohol</th>
<th>Measure</th>
<th>Unit</th>
</tr>
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<tbody>
<tr>
<td>Ordinary strength larger (4%) e.g. Carling, Fosters</td>
<td>Pint</td>
<td>2.3</td>
</tr>
<tr>
<td>Strong larger (5.2%) e.g. Stella Artois, Kronenburg</td>
<td>Pint</td>
<td>3</td>
</tr>
<tr>
<td>Strong large e.g. Stella Artois, Carlsberg Export, Grolsch</td>
<td>440ml can</td>
<td>2.2</td>
</tr>
<tr>
<td>Beer/ordinary strength Ale e.g. John Smith’s, Guinness</td>
<td>Pint</td>
<td>2.3</td>
</tr>
<tr>
<td>Red/White Wine</td>
<td>Std 175ml</td>
<td>2</td>
</tr>
<tr>
<td>Red/White Wine</td>
<td>Lg.250ml</td>
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<tr>
<td>Spirits</td>
<td>Std 25ml</td>
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<tr>
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<td>1.4</td>
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<tr>
<td>Alcopop e.g. Smirnoff Ice, Bacardi Breezer, Reef</td>
<td>275ml</td>
<td>1.5</td>
</tr>
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</table>

Signed ___________________________       Date ____/ ____/______
Appendix 11

PARTICIPANT INFORMATION SHEET:

PROJECT TITLE: Assessment of the effect of a daily cinnamon supplement on the inflammatory markers, lipid profiles, glucose tolerance and insulin response

INSTITUTION: Department of Nutritional Sciences, Faculty of Health and Medical Sciences, University of Surrey

INVESTIGATORS: Tahany Al-Dayel, Dr Jonathan Brown, Professor Susan Lanham-New

INTRODUCTION

We would like to invite you to take part in a research project. Before you decide you need to understand why the research is being done and what it will involve for you. Please take the time to read the following information carefully. Talk to others about the study if you wish.

BACKGROUND

Metabolic syndrome is a disorder of a collection of conditions that occur together, including high blood pressure, insulin resistance, excess body fat around the waist, high cholesterol and high fasting glucose. All these features are risk factors for cardiovascular disease, type 2 diabetes and kidney disease. The risk of having metabolic syndrome is closely linked to being overweight or having obesity. Furthermore, adults with metabolic syndrome show a higher inflammatory state, which also increases with age. Inflammation is generally a protective mechanism produced in humans to remove injurious or harmful stimuli from body tissue and this is part of a healthy immune response. However, although inflammation can occur acutely (as above) it can also be produced chronically and it can lead to the development of several chronic diseases such as cancer, diabetes, heart disease and Alzheimer’s disease. The level of inflammation in the body can be measured by assessing the level of inflammatory markers. Thus, high levels of inflammatory markers are associated with most chronic diseases such as diabetes.

Many herbal products are now available that claim to have health beneficial properties. Cinnamon (Cinnamomum cassia) is an example of this that has been claimed to improve blood glucose, insulin levels and blood lipid profile. There is also some evidence that it can decrease inflammation in human studies. Furthermore cinnamon is a traditional medicine used in the treatment of dyspepsia, gastritis, blood circulation issues and inflammatory disease. Cinnamon has many pharmacological properties such as being an anti-inflammatory agent, an anti-diabetic agent as well as having anti-tumor activity. The aim of this study is to investigate whether consuming 5 g of a cinnamon supplement (Cinnamomum cassia) every day for two months will improve inflammatory markers, lipid profiles, glucose tolerance and insulin response.

YOU CAN ONLY TAKE PART IN THIS STUDY IF YOU ARE:

4. Female aged 45-70 years
5. Free from medication
6. Free from cinnamon allergy
7. Overweight or obese (BMI≥25)
8. Not taking supplements in last 1 months
9. Non-smokers
10. Healthy and free from any disease
11. Able and willing to comply with all study procedures

YOU CANNOT TAKE PART IN THIS STUDY IF YOU:
9. Have a personal history of psychiatric disorders, epilepsy, sleep disorders, diabetes, CHD or hypertension, liver disorder or food allergies
10. Have suffered from infectious hepatitis, jaundice, malaria, tuberculosis and are not HIV positive
11. Have a history of drug or alcohol abuse
12. Have taken any medication (e.g. either a prescription medicine (e.g. antibiotics) or an over the counter medicine other than vitamin supplements
13. Have any medical problems
14. Have received an experimental drug either clinically or recreationally within the past 1 months
15. Have a phobia of needles/sight of blood
16. Have a cinnamon allergy
17. Already taking cinnamon or other herbal supplement
18. Participated in a clinical trial within the last 1 months
19. Have an addiction to caffeine products (more than 3 cups of caffeinated products per day)

STUDY PROCEDURE
Once you have shown an interest to be involved in this study, you will then be asked to answer a screening questionnaire regarding your current health and lifestyle. To make sure you are suitable to enroll in this study we will check your blood pressure and fasting blood glucose by finger prick test. You will be offered an appointment to attend the Clinical Investigation Unit (CIU) 17AX00 at the Faculty of Health and Medical Sciences after an overnight fasting which means you should not consume anything except water from 20:00 h prior to the screening days. Refreshments thought will be offered after the screening day.

The screening days will help us decide whether you are eligible and healthy to take part in the study. It also helps us make sure that it is safe for you to be involved in the study (e.g. some health conditions will mean you cannot take part). If you are eligible for the study then you will be offered an appointment to attend the Clinical Investigation Unit (CIU) 17AX00 at the Faculty of Health and Medical Sciences, for your first visit session. All information obtained from the study will be restricted to the investigators only and will be kept strictly confidential.

This study has received a favourable ethical opinion from the University of Surrey Ethics Committee. All participates must sign a consent form before they participate in the study.

In this study you will receive capsules to consume every day for 8 weeks. These capsules may contain cinnamon or not contain cinnamon (placebo). You will consume 5 g of the cinnamon (that has been purchased from Puritan’s Pride) or placebo as a capsule. Three capsules (the total will be 2.5 g) after your breakfast meal and three capsules (the total will be 2.5g in the evening) after your evening meal.

Please do not squeeze or open the capsules.
STUDY VISITS

You will be asked to be involved in the study for a period of 8 weeks. During this time, you will be required to visit the CIU on three separate occasions over the 8 weeks.

Visits will take place at Week 0, Week 4 and Week 8.

All three visits will follow a very similar order of activities. Each visit will last approximately 3 hours and all visits will take place in the morning (8 am to 11 am). For the purposes of obtaining an appropriate blood sample (required as part of the study), you will be requested to attend the CIU after an overnight fast, which means you should not consume anything except water from 20:00 h (8 pm) prior to each of the study days. However, once you have completed all of the assessments on each study visit day you will be offered refreshments.

The study activities for each visit will be as follows:

Visit 1 (Week 0)
- A blood sample (approximately 25 ml) will be taken in the fasted state.
- You will then be asked to drink a glucose drink (75 g of glucose), and then after 2 hours, another blood sample (approximately 5 ml) will be taken for the oral glucose tolerance test (OGTT).
- Your anthropometric measurements including height, weight, body composition, (measured by bioelectrical impedance analysis), waist and hip circumference, heart rate and blood pressure will be determined.
- A scan of your wrist bones by a technique called Peripheral Quantitative Computed Tomography (PQCT) will be completed.
- Diet Diary before your first visit; you will have been asked to record all your food intake for three days prior the study date. This information form will be collected from you at your visit.
- Refreshments (e.g. coffee, tea, juice) and standard breakfast will be offered and you will then be supplied with cinnamon or placebo capsules that you have been randomly assigned to consume for the study.

Visit 2 (Week 4)
- This visit will be identical to study visit 1 except the oral glucose tolerance test (OGTT) and the scanning by PQCT, will be not be required.
- Blood samples (approximately 25 ml), anthropometric measurements and blood pressure will be repeated as described for Visit 1.
- Diet Diary before your second visit; you will have been asked to record all your food intake for three days prior the study visit date. This information form will be collected from you at your visit.
- Time available for you to talk with us about the study and how you have found the supplement.
- Refreshments (coffee, tea) and standard breakfast will be offered (as per study visit 1).

Visit 3 (Week 8)
- This visit will be identical to visit 1 except the scanning by PQCT will not be required.
- A blood sample (approximately 25 ml) will be taken in the fasting state.
- You will be asked to drink a glucose drink (75 g of glucose), and then after 2 hours, another blood sample (approximately 5 ml) will be taken for the oral glucose tolerance test (OGTT).
- Your anthropometric measurements (including height, weight, body composition (measured by bioelectrical impedance analysis), waist and hip circumference, heart rate and blood pressure will be determined.
- Diet Diary before your third visit; you will have been asked to record all your food intake for three days prior the study date. This information form will be collected from you at your visit.
- Refreshments (coffee, tea, juice) and standard breakfast will be offered and you will be asked to return the remaining supplements. You will now have completed the study.

PROCEDURES TO BE FOLLOWED BEFORE THE STUDY VISIT

For 3 days prior to each study day, you must refrain from intense physical exercise and refrain from consuming alcohol. Moreover, you must refrain from consuming caffeine-containing drinks (tea/coffee/carbonated soft drinks such as Pepsi, Coco-cola, Red Bull) for 24 hours before each visit. Drinking caffeine products might affect the blood glucose levels which are an important part of the study so we want you to try to avoid these drinks 24 hours before each study visit day. Dietary food intake records will be completed by you to provide information on your dietary intake for 3 days before each study day (you will need to write down all that you have consumed (food and drink) over a 3 day period before each study visit in as much detail as possible). These will be used to standardize dietary intake before the oral glucose tolerance test OGTT trials, which will be a part of the study test measurements. In addition, all participants will be fed the same standardized meal (which will be a vegetable lasagna (400 g) the evening before each of the study days. You will not be allowed to consume any food or drink, with the exception of water, from 20:00 h prior to each of the study days.

NUMBER OF PARTICIPANTS EXPECTED TO PARTICIPATE

You will be one of approximately 46 subjects who will participate in this study

GENERAL

All information and data obtained from this study will be restricted to the investigators only and will be kept strictly confidential. The data obtained from all participants will be used to establish the effect of cinnamon on inflammatory markers, lipid profiles, glucose tolerance and insulin response. Once the study is completed a summary of the work will available to each participant on request. It is hoped that the work, which will be anonymised, will also be published at a conference. You will be free to withdraw from the study at any time, without needing to justify your decision and without prejudice.

ANALYSIS

Blood samples collected during the study will be analysed for inflammatory markers, lipid profiles, glucose and insulin response. All samples will be analysed at the appropriate laboratory. There is no DNA analysis in this study and the remaining blood samples will be destroyed at the end of the study according to the University rules for the disposal of biological materials.

PAYMENT FOR PARTICIPATION

We will pay the public transportation costs associated with your travel to the University on screening and visit days or the cost of car parking. You will also receive £100 for completing the study. These payments will be made at the end of study.

DISCOMFORT AND RISKS ASSOCIATED WITH STUDY
The risk of side effects is likely to be minimal. However, you may experience slight discomfort when swallowing the capsules every day for 8 weeks. You will have a chance to speak to us at visits 2 and 3 to discuss any issues you may be having, and contact us via telephone or email throughout the study if your enquiry needs to be answered more quickly.

Blood samples will be taken by a fully trained phlebotomist/nurse at each trial visit. To minimize infection they will wash their hands thoroughly and use disinfectant rubs before taking blood from each participant. Taking the blood sample may cause some slight bruising and this may take some time to go away. If the bruising continues we will advise you to go to your GP. Occasionally some people may feel faint when they have their blood taken and so to help us reduce the risk of this, please let us know if you have any phobia of needles/sight of blood. Nevertheless, the staff in the CIU are fully trained to deal with such situations. It is the participant’s responsibility to inform the investigators if you feel dizzy, ill or have other symptoms before and during blood donation. Your blood sample will be taken in a reclined chair by qualified phlebotomist/nurse.

Caffeine withdrawal symptoms may occur when a person misses their daily dose of caffeine (such as tea, coffee or cola). The common symptoms are headache and lack of concentration. In these cases, we advise you to be aware of these symptoms.

Peripheral Quantitative Computed Tomography (PQCT) scan involves an extremely low level of ionising radiation which you will be exposed to once during the course of the study. The amount of radiation absorbed from the scan is 0.5μ Sv which is equivalent to two hours of natural background radiation found in the UK which is 2.2 Sv per year.

Ionising Radiation Medical Exposure Regulations (IRMER)

A peripheral Quantitative Computed Tomography (PQCT) scan of the forearm will be performed at the University of Surrey. The procedure is painless and does not involve going through a tunnel of any kind. The radiation dose is considered negligible and is equivalent to around 2 hours natural background radiation that we are exposed to throughout our lives: less than 1/10 of a normal chest X-ray. You will be asked to sit on a seat where a scan of your forearm will be performed. The procedure involves placing your forearm into the scanning machine where an X-ray beam moves across it. The scans together take approximately 20 minutes to complete and must be removed for the arm scan.

CONTACT DETAILS

If you should have any questions regarding any aspect of this study, please contact: Tahany Aldayel (e-mail: T.Aldayel@surrey.ac.uk) or Dr Jonathan Brown (e-mail: J.E.Brown@surrey.ac.uk).

Any complaint about any aspect of this study you can contact Dr Jonathan Brown (e-mail: J.E.Brown@surrey.ac.uk).

Thank you for taking time to read this Information Sheet
Appendices

Appendix 12

Consent Form:

- I the undersigned voluntarily agree to take part in the study on Assessment of the effect of a daily cinnamon supplement on the inflammatory markers, lipid profiles, glucose tolerance and insulin response.
- I have read and understood the Information Sheet provided. I have been given a full explanation by the investigators of the nature, purpose, location and likely duration of the study, and of what I will be expected to do. I have been advised about any discomfort and possible ill-effects on my health and well-being which may result. I have been given the opportunity to ask questions on all aspects of the study and have understood the advice and information given as a result.

- I agree to follow all the instruction that given to me during the study and to co-operate fully with the investigators. I shall inform them immediately if I suffer any deterioration of any kind in my health or well-being, or experience any unexpected or unusual symptoms.

- I consent to my personal data, as outlined in the accompanying information sheet, being used for this study and other research. I understand that all personal data relating to volunteers is held and processed in the strictest confidence, and in accordance with the Data Protection Act (1998).

- I am healthy and have disclosed any known medical problems and I am not taking medicines and supplements.

- I understand that I am free to withdraw from the study at any time without needing to justify my decision and without prejudice.

- I acknowledge that in consideration for completing the study I shall receive the cost of public transportation or car parking.
- I understand that in the event of my suffering a significant and enduring injury (including illness or disease) as a direct result of my participation in the study, compensation will be paid to me by the University (or sponsor where a clinical trial is sponsored by a pharmaceutical company), subject to certain provisos and limitations. The amount of compensation will be appropriate to the nature, severity and persistence of the injury and will, in general terms, be consistent with the amount of damages commonly awarded for similar injury by an English court in cases where the liability has been admitted.

- I confirm that I have read and understood the above and freely consent to participating in this study. I have been given adequate time to consider my participation and agree to comply with the instructions and restrictions of the study.

- I acknowledge that in consideration for completing the study I shall receive £100. I recognise that the sum would be less, and at the discretion of the Principal Investigator, if I withdraw before completion of the study.
Appendices

Name of volunteer (BLOCK CAPITALS) ........................................................
Signed ........................................................
Date ........................................................

Name of witness (where appropriate) (BLOCK CAPITALS)
........................................................
Signed ........................................................
Date ........................................................

Name of researcher/person taking consent (BLOCK CAPITALS) ........................................................
Signed ........................................................
Date ........................................................
Appendices

Appendix 13

Conference and publications


