The Effects of Coffee on Glucose Metabolism

by

Tracey M. Robertson

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Faculty of Health and Medical Sciences
University of Surrey

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Declaration of originality

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Summary

It has been suggested that coffee drinking may confer a beneficial effect on health by reducing the risk of developing Type 2 Diabetes Mellitus (T2DM) and indeed there is much epidemiological evidence for a reduced incidence of T2DM in habitual coffee drinkers. However, many acute studies have reported a temporary worsening in postprandial glycaemia following caffeinated coffee (CC) consumption. Varied methodologies have been employed by these studies with many giving their participants large doses of coffee.

In the acute studies conducted for this thesis, a single serving of CC increased the postprandial glycaemic response more than control (p=0.008), with no apparent dose-response effect. Furthermore, a single serving of decaffeinated coffee (DC) consumed in the morning, produced no effect on postprandial glycaemia, although a trend was observed for a reduction in the postprandial glucose peak (p=0.060) when DC was consumed at lunchtime.

The majority of longer-term investigations have recruited habitual coffee drinkers who are likely to have already obtained any potential benefits of coffee consumption. The longer-term intervention reported in this thesis found no overall effects of twelve weeks of CC drinking on glucose and lipid metabolism in coffee-naïve individuals. However, differences were observed between fast and slow caffeine metabolisers when the analysis was split by phenotype. The fast caffeine metabolisers displayed a lower postprandial glucose response (p=0.019) and greater NEFA suppression (p=0.001) at baseline. Furthermore, significant interaction effects were observed between visit and phenotype for postprandial glucose (p=0.048) and NEFA (p=0.019), with the intervention producing an apparent increase in postprandial glycaemia in fast metabolisers and reduced NEFA suppression in slow metabolisers.

In conclusion, no evidence was found for a beneficial effect of coffee drinking on glucose and lipid metabolism in the general population, however, individual differences in response to longer-term coffee drinking were observed which warrant further investigation.
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**Abbreviations**

- AMPK: 5’ adenosine monophosphate-activated protein kinase
- ANOVA: Analysis of variance
- ATGL: Adipose triacylglycerol lipase
- AUC: Area under curve
- BMI: Body mass index
- BP: Blood pressure
- BW: Bodyweight
- cAMP: Cyclic adenosine monophosphate
- CC: Caffeinated coffee
- CFQA: Caffeoylferuloylquinic acid
- CGA: Chlorogenic acid
- CHO: Carbohydrate
- CIU: Clinical Investigation Unit
- CQA: Caffeoylquinic acid
- CRP: C-reactive protein
- DBP: Diastolic blood pressure
- DC: Decaffeinated coffee
- DF: Dietary fibre
- DHCA: Dihydrocaffeic acid
- DHFA: Dihydroferulic acid
- diCQA: Dicaffeoylquinic acid
- diFQA: Diferuloylquinic acid
- DR: Dark roast
- DXA: Dual-energy X-ray absorptiometry
- ELISA: Enzyme-linked immunosorbent assay
- EPIC: European Prospective Investigation into Cancer
- FA: Fatty acid
- FFA: Free fatty acids
- FQA: Feruloylquinic acid
- G1P: Glucose 1-phosphate
- G3P: Glycerol-3-phosphate
- G6P: Glucose 6-phosphate
- G6Pase: Glucose 6-phosphatase
- GCE: Green coffee extract
- GI: Glycaemic index
GIP  Glucose-dependent insulinotropic polypeptide
GIR  Glucose infusion rate
GLP-1 Glucagon-like peptide-1
GLP-2 Glucagon-like peptide-2
GLUT Glucose transporter
HbA1c Glycated haemoglobin
HDL High density lipoprotein
HMW High molecular weight
HOMA Homeostasis Model of Assessment
HPLC High performance liquid chromatography
HSL Hormone sensitive lipase
IAUC Incremental area under curve
IFG Impaired fasted glucose
IGT Impaired glucose tolerance
IL-18 Interleukin-18
IL-6 Interleukin-6
IR Insulin resistance
LDL Low density lipoprotein
LMW Low molecular weight
LPL Lipoprotein lipase
LR Light roast
MAG Monoacylglycerol
MAGL Monoacylglycerol lipase
MGB Minor groove binder
MLR Medium light roast
MR Medium roast
MS Metabolic Syndrome
NEFA Non-esterified fatty acid
NFQ Nonfluorescent quencher
OGTT Oral glucose tolerance test
pCoQA p-coumaroylquinic acid
PCR Polymerase chain reaction
PKA Protein kinase A
PNS Parasympathetic nervous system
QC Quality control
RDS Rapidly digestible starch
RIA Radioimmunoassay
RM Repeated measures
RR Relative risk
RS Resistant starch
SBP Systolic blood pressure
SCRC Surrey Clinical Research Centre
SD Standard deviation
SEM Standard error of the mean
SGLT Sodium-glucose linked transporter
SNP Single nucleotide polymorphism
T2DM Type 2 Diabetes Mellitus
TAG Triacylglycerol
TNF-α Tumour necrosis factor alpha
VLDL Very low density lipoprotein
WHO World Health Organisation
Chapter 1. Introduction

1.1 Overview

Diabetes is a global health problem, with the World Health Organisation (WHO) estimating the prevalence to be 9% of the adult population in 2014 \(^1\). It affects almost 350 million people worldwide, the majority of which (90%) have T2DM. If poorly controlled, it can result in serious complications, including cardiovascular disease, nephropathy and retinopathy, and it is estimated to have caused 1.5 million deaths in 2012 \(^1\).

There is much epidemiological evidence demonstrating a reduced risk of T2DM in coffee drinkers, particularly in those who drink large amounts of coffee, with a reduced risk being reported for both caffeinated and decaffeinated coffee drinkers \(^2–6\). However, it is not known precisely how coffee may exert this protective effect. Furthermore, studies into the acute effects of CC have generally reported a detrimental effect on the postprandial glycaemic response compared to a control \(^7–9\). Caffeine in isolation has been demonstrated to produce a greater increase in the postprandial glycaemic excursion than that observed following CC ingestion \(^10\), giving rise to the hypothesis that components in coffee, other than caffeine, may be attenuating the acute detrimental effects of caffeine. There have been few longer-term interventions into the effects of coffee on glucose metabolism, the majority of which have examined the effects in habitual coffee drinkers \(^11–13\).

This chapter will begin with an overview of glucose and lipid metabolism and how it is affected by T2DM. It will then discuss coffee production and the various components of coffee and will end with a review of the literature on coffee’s effects on glucose and lipid metabolism.

1.2 Glucose homeostasis

Glucose is the main fuel for the brain due to its ability to cross the blood-brain barrier. Blood glucose is typically about 5 mmol/L after an overnight fast, dropping further to approximately 3.0 mmol/L under conditions of prolonged fasting \(^14\). In the postprandial state, in healthy individuals, it rarely exceeds 7.8 mmol/L \(^15\). High blood glucose over a prolonged period, such as can be found in poorly controlled T2DM, can lead to serious macro- and microvascular problems \(^16\). It is therefore very important for the body to maintain blood glucose levels within a relatively narrow range, both to prevent
the complications associated with high levels and to ensure sufficient fuel for the brain. For healthy individuals, it is also important to minimise the postprandial glucose excursion as elevated postprandial glycaemia is associated with development of T2DM. In the fasted state, plasma glucose levels are maintained by glycogenolysis (breakdown of glycogen to glucose in the liver) and gluconeogenesis (formation of glucose in the liver and kidney from other compounds such as lactate and pyruvate). In the postprandial state, plasma glucose originates mainly from the digestion and absorption of dietary carbohydrate (CHO), although hepatic glucose production is never completely switched off and continues to make a small contribution. Glucose is taken up from the blood into the various organs by two families of transporters: the GLUT family (by passive diffusion) and the SGLT family (by active transport), by both insulin-mediated and independent processes. The rates of appearance and clearance of glucose determine plasma glucose concentrations at any given time. These are regulated either directly or indirectly by a number of hormones such as insulin, glucagon, cortisol, adrenalin, glucose-dependent insulinotropic polypeptide (GIP), glucagon-like peptide-1 (GLP-1) and growth hormone, the relative importance of which depends on whether the body is in the fasted or postprandial state. These regulatory mechanisms will be covered in more detail later.

1.2.1 Postprandial glucose metabolism

1.2.1.1 Carbohydrate digestion

Dietary CHO consists of simple sugars such as the disaccharides, sucrose and lactose, and complex CHOs such as rapidly digestible starch (RDS), resistant starch (RS) and dietary fibre (DF). RS and DF pass through the small intestine undigested to the colon and will not be discussed further here. RDS consists of the polysaccharides amylose and amylpectin: long, unbranched (amylose) and branched (amylpectin) chains of glucosyl units which are digested in a multi-stage process by enzymes in the mouth and gut. Starch digestion begins in the mouth, where the α-amylase enzyme, secreted in saliva, hydrolyses α-1,4 bonds anywhere in the chain, breaking the starch down into smaller units. This continues in the upper stomach for some time, before the acidic environment of the stomach inhibits the process. The process is finished in the lumen.
of the small intestine by $\alpha$-amylase secreted in pancreatic juices, resulting in small oligosaccharides and di- and trisaccharides, maltotriose and maltose.

These smaller molecules, along with the simple sugars, are hydrolysed by brush border enzymes, $\alpha$-glucosidase, sucrase, maltase and lactase, to the monosaccharides glucose, fructose and galactose, ready for absorption\(^ {26}\). The absorption and disposal of fructose and galactose will not be covered in this thesis.

### 1.2.1.2 Glucose absorption

Glucose is absorbed into the enterocyte via two different transporters on the apical membrane. When glucose concentrations in the lumen are high, GLUT2 transporters translocate to the apical membrane and glucose diffuses across them\(^ {27}\). When glucose levels are lower, the GLUT2 transporters are recycled and glucose is co-transported with Na\(^+\), against the concentration gradient, by the SGLT1 transporter via an active transport mechanism. Glucose then enters the circulation via GLUT2 transporters in the basolateral membrane.

### 1.2.1.3 Glucose disposal

Absorbed glucose is taken by the portal vein to the liver where it enters via GLUT2 transporters. Approximately 30% of absorbed glucose is retained by the liver and stored as glycogen; the remainder passes into the systemic circulation. Of the glucose entering the circulation, about 20% is taken up into the brain via GLUT1 transporters, about 40% is taken up by skeletal muscle via GLUT4 transporters and the remainder is either taken up by the kidneys (GLUT2) and adipose tissue (GLUT4) or returned to the liver\(^ {28}\).

### 1.2.2 Glucose production: gluconeogenesis and glycogenolysis

Glucose can be produced by the liver from various substrates, the main ones being alanine, lactate and glycerol, in a process called gluconeogenesis. It is a multi-step process involving the formation of several intermediaries, with the terminal step being the conversion of glucose 6-phosphate (G6P) to glucose, catalysed by the glucose 6-phosphatase enzyme (G6Pase). Glucose can also be produced by the breakdown of glycogen stores in the liver, in a process called glycogenolysis. In this process glycogen is broken down to glucose 1-phosphate (G1P), which converts to G6P and thereafter to glucose as in gluconeogenesis. Whilst these processes occur mainly in the fasted state, they are not entirely inhibited in the postprandial period.
1.2.3 Lipid metabolism

Lipids are the body’s main source of energy in the fasted state. They are stored in adipose tissue as triacylglycerol (TAG). When required, they are hydrolysed to free fatty acids (FFA) and glycerol by the actions of adipose triacylglycerol lipase (ATGL), hormone-sensitive lipase (HSL) and monoacylglycerol lipase (MAGL) in a process called lipolysis (29). They are then released into the circulation along with the liberated glycerol. The FFAs which are released are non-esterified fatty acids (NEFAs) and are transported in the blood bound to albumin. NEFA levels tend to be highest in the morning and decrease after a meal.

After a meal, digestion of dietary lipid begins in the stomach through the actions of gastric lipase. When the partially hydrolysed lipid arrives in the small intestine it is emulsified by bile salts released from the gall bladder. Pancreatic lipase further hydrolyses the lipids resulting in fatty acids (FAs) and monoacylglycerols (MAGs) which are packaged into micelles, along with bile salts, for transportation through the intestine. FAs and MAGs are absorbed into the enterocyte via transporter proteins. Once inside the cell they are re-esterified to TAG and are packaged, along with proteins and phospholipids, into chylomicrons in which they are transported, via the lymphatic system, into the blood (30). Absorption of lipid is a lengthy process with peak TAG levels occurring about 3 - 5 h after a meal.

As the chylomicrons travel through the blood they interact with other particles such as high density lipoproteins (HDL), transferring apolipoproteins between them. As they pass tissues such as muscle and adipose tissue they come into contact with lipoprotein lipase (LPL) on the endothelial surface of these tissues. LPL hydrolyses TAG, releasing NEFAs which are taken up into the cells and either used immediately for energy or repackaged into TAGs for storage (31). LPL requires the presence of apolipoprotein CII, which the chylomicron acquires from HDL, in order to release TAG from it. As the chylomicrons travel around the body they reduce in size as more and more TAGs are liberated from them, eventually becoming chylomicron remnants which are taken up by the liver (29).

TAGs are also released into the blood from the liver in very low density lipoproteins (VLDL). These VLDL also interact and exchange contents with other particles, such as HDL, and deliver TAG to the tissues via the actions of LPL, much like chylomicrons. They also reduce in size, becoming cholesterol-ester-rich low density lipoproteins (LDL), which deliver cholesterol to the tissues. Some LDL remains in the circulation and some is taken up by the liver. HDL is also released by the liver; its main function
is to accept cholesterol from the tissues and transport it back to the liver where it is converted to bile salts or excreted in bile.

### 1.2.4 Regulation of glucose and lipid metabolism

#### 1.2.4.1 Insulin

When food is seen, smelled or tasted the parasympathetic nervous system (PNS) stimulates the release of saliva; it also triggers first-phase release of insulin. In the fed state, when blood glucose concentrations are raised, glucose binds to receptors on the pancreas and signals the β cells of the pancreas to increase insulin release. Insulin secretion is further stimulated by the insulin potentiatiors, GIP and GLP-1. GIP is secreted by the K cells of the proximal intestine in response to nutrients in the gut; its concentration is strongly correlated with the rate of glucose absorption \(^{(32)}\). GIP also stimulates the L cells of the intestine to release glucagon-like peptide-2 (GLP-2), which has been demonstrated to promote the translocation of GLUT2 receptors to the apical membrane thereby increasing glucose absorption \(^{(27)}\). GLP-1 is secreted by the L cells of the distal intestine in response to glucose presence in the gut. It has been estimated that the actions of incretins contribute between 20 and 70% of the total insulin response after oral glucose administration \(^{(33,34)}\).

Insulin acts in a variety of ways, both directly and indirectly, to reduce plasma glucose concentration. It binds to receptors on muscle and adipose tissue, activating signalling pathways, leading to translocation of glucose transporters to the cell surface and thus enhancing glucose uptake into these tissues. It suppresses the action of HSL, reducing the amount of NEFA released into the circulation, which in turn reduces the amount of gluconeogenesis. It also promotes glycolysis (the storage of glucose as glycogen) by inhibition of some of the key enzymes involved in the breakdown of glycogen, such as G6Pase, and by stimulating glycogen synthase, an enzyme involved in glycogen synthesis \(^{(35)}\).

#### 1.2.4.2 Glucagon

Glucagon is a counter-regulatory hormone to insulin and is released from the α cells of the pancreas when glucose levels are low. It is mainly inhibited by high blood glucose and insulin, but also by GIP and GLP-1 \(^{(33)}\). Its main action is to increase blood glucose. It binds to G-protein coupled receptors (GPCRs) on its target cells activating a signalling cascade involving formation of cyclic adenosine monophosphate (cAMP).
and activation of protein kinase A (PKA). In hepatic tissue this leads to release of glucose by glycogenolysis.

1.2.4.3 Adrenalin

Adrenalin is a catecholamine secreted by the adrenal medulla in times of stress. It binds to adrenoreceptors (GPCRs), of which there are two groups: α- and β-receptors. Adrenalin has different effects on glucose metabolism, depending on which receptor it binds to. The receptors are present in varying proportions on different tissues and it is the relative proportion of each that determines the overall effect of adrenalin on that tissue.

Binding to β-receptors results in stimulation of cAMP and activation of PKA, resulting in increased glycogenolysis and gluconeogenesis, thus raising blood glucose in the same way as glucagon. In vitro work has demonstrated that cAMP also stimulates glucose transport across both the brush border and basolateral membranes (36).

Stimulation of cAMP in adipose tissue leads to activation of HSL by phosphorylation which causes the release of NEFA and glycerol, which can be utilised in gluconeogenesis. Binding to α2-receptors inhibits cAMP, thereby counteracting the effects of the β-receptors, particularly with respect to the mobilisation of fatty acids in adipose tissue. Binding to α1-receptors activates a process which increases cytosolic Ca²⁺, which in turn activates PKA leading to increased blood glucose. Adrenalin also inhibits glucose uptake in skeletal muscle via the β2-receptor (37). Although α- and β-receptors can have opposing actions, the overall net effect of adrenalin binding to them is to raise blood glucose.

1.2.4.4 Cortisol

Cortisol is a glucocorticoid secreted by the adrenal cortex, which is increased in times of stress. Its levels change throughout the day and are highest in the morning. It has a similar effect to adrenalin on glucose metabolism but its effects are not immediate as it works mainly by affecting protein synthesis rates of key enzymes (38). The net result of increased cortisol is an increase in HSL activity, leading to release of NEFA and glycerol from adipose tissue and increased gluconeogenesis in the liver. It has also been demonstrated to reduce glucose uptake in skeletal muscle and impair insulin release from the pancreas.
1.2.4.5 Inflammatory markers

Tumour necrosis factor alpha (TNF-\(\alpha\)) and interleukin-6 (IL-6) are adipocytokines, released by adipose tissue as part of the inflammatory response. They also play a role in glucose regulation. TNF-\(\alpha\) inhibits insulin receptor tyrosine kinase activity thus inhibiting glucose uptake and increasing insulin resistance in adipose and muscle tissue \(^{(39)}\). TNF-\(\alpha\) and IL-6 both inhibit LPL thus increasing circulating NEFA, raised levels of which are associated with insulin resistance and obesity \(^{(40)}\).

1.2.4.6 Adiponectin

Adiponectin is a hormone which is mainly secreted by adipose tissue. It circulates in the blood in several forms: as dimers, trimers or as protein complexes of high molecular weight (HMW) and low molecular weight (LMW) hexamers. Although it is secreted by adipose tissue, circulating levels are inversely associated with adipose tissue mass, with lean people generally having higher levels than the obese \(^{(41)}\). Adiponectin increases insulin sensitivity by several methods. It binds to receptors on skeletal muscle, activating 5' adenosine monophosphate-activated protein kinase (AMPK), increasing translocation of GLUT4 to the cell surface and resulting in greater glucose uptake \(^{(42)}\). It also inhibits gluconeogenesis via inhibition of one of the key enzymes in the pathway, phosphoenolpyruvate carboxylase \(^{(43)}\).

1.2.5 Alterations to glucose homeostasis in T2DM

T2DM is a disease characterised by high fasted blood glucose and impaired insulin sensitivity; obesity and Metabolic Syndrome (MS) are strong risk factors. According to the WHO \(^{(44)}\), a person has MS if they have insulin resistance, determined by one of:

- T2DM (fasted glucose \(\geq 7.0\) or 2 h glucose \(\geq 11.1\) mmol/L)
- Impaired fasted glucose (fasted glucose 6.1 – 6.9 mmol/L)
- Impaired glucose tolerance (2 h glucose 7.8 – 11.0 mmol/L)

Plus any two of:

- High blood pressure (systolic \(\geq 140\) mm Hg or diastolic \(\geq 90\) mm Hg)
- Plasma TAG \(\geq 1.7\) mmol/L
- HDL cholesterol < 0.9 mmol/L in men or < 1.0 mmol/L in women
- Body mass index (BMI) > 30 kg/m\(^2\) and/or waist:hip ratio >0.90 in men and >0.85 in women
High fasted NEFA levels are associated with development of T2DM\(^{(45,46)}\), however a re-analysis of data from three cohort studies concluded that this association disappears when 2 h glucose is included in the analysis\(^{(47)}\). Furthermore another prospective cohort study found no association between baseline fasted NEFA and development of glucose intolerance, but did find an association between fasted NEFA and glucose intolerance when they performed a cross-sectional analysis on their data, leading them to conclude that NEFA levels change alongside development of MS, rather than MS developing as a consequence of this lipotoxicity\(^{(48)}\). Many people with poorly controlled T2DM also have elevated NEFA\(^{(49)}\), although a meta-analysis of studies, examining the relationship between NEFA and obesity, failed to find an association between fat mass and NEFA levels\(^{(50)}\). High intra-individual variation in NEFA has been found with day-to-day variation being as high as 45%\(^{(51)}\), with NEFA levels being particularly sensitive to stress states. This daily variation is likely to confound results of studies examining associations between NEFA and T2DM and obesity. Chronically high NEFA leads to impaired insulin sensitivity and reduced insulin secretion from the pancreas\(^{(52)}\). Acutely elevated NEFA reduces insulin sensitivity via reduced glucose transport in muscle\(^{(53)}\). Reduced insulin sensitivity results in failed suppression of NEFA release and reduced uptake of TAG into cells, causing greater circulating NEFA. As insulin resistance increases, the pancreas initially produces more insulin in order to regulate blood glucose. This can lead to failure of pancreatic \(\beta\) cells to respond to glucose and reduced insulin secretion resulting from both loss of \(\beta\) cell mass and amyloid deposition.

Various studies have reported associations between inflammatory markers and T2DM. A recent meta-analysis found a dose-response association between IL-6 levels and risk of T2DM\(^{(54)}\). Furthermore, an analysis of data from the Nurses' Health Study found significant associations between baseline levels of TNF-\(\alpha\) receptor 2, IL-6 and C-reactive protein (CRP) and T2DM risk. However, when all three markers were analysed together, only the association with CRP remained\(^{(55)}\). Increased levels of the pro-inflammatory cytokines, TNF-\(\alpha\) and IL-6 are also evident in obesity.

Adiponectin levels tend to be lower in T2DM and MS\(^{(56)}\), although this may not be a consequence of the disease as there appears to be a genetic predisposition to lower adiponectin levels with associations having been found between various polymorphisms in the adiponectin gene and lower circulating adiponectin levels and T2DM risk\(^{(57)}\).
1.3 Coffee

1.3.1 Coffee consumption in the UK

Coffee consumption in the UK is growing rapidly with coffee imports to the UK increasing by 45% between 2004 and 2014 (58). Most of the coffee imported to the UK in 2013 was in the form of green coffee beans (2.5 million x 60 kg bags), followed by instant coffee (1.2 million x 60 kg bags) and roasted coffee (0.5 million x 60 kg bags). The UK population, as a whole, consumed 174 million kg of coffee in 2014, equivalent to 2.8 kg per capita (59). Instant coffee is the most popular form of coffee in the UK and accounted for almost 75% of retail value sales in 2015 (60). However, its popularity has been in decline in recent years, as sales of pod-type coffee increases, with the overall percentage of people drinking instant coffee dropping from 70% in 2014 to 64% in 2015 and only 35% drinking it at least once a day. Nevertheless, this is still substantially higher than the 15% of people who drink ground coffee at least once per day (60).

1.3.2 Coffee production

More than 80 species of coffee have been identified (61), including several which are naturally caffeine-free, such as Coffea bengalensis (62), however the majority are not commercially important. Only two species, C. arabica and C. camphora, account for the majority of global sales. Arabica coffee accounts for over 65% of coffee exports with C. camphora, also known as Robusta coffee, comprising the remainder (63). Arabica beans are generally considered to produce a better quality coffee and are most commonly used in the preparation of filter and espresso-type coffees, whereas Robusta beans are favoured in the production of instant coffee.

After harvesting, the outer pulp is removed from the ripe coffee berries to produce the green coffee beans. The pulp is removed in one of two ways, either by soaking in water or by air drying followed by mechanical abrasion of the dried outer shell. The wet method is considered to produce a superior product. After the green beans have been dried they are ready for decaffeination or roasting.

There are several methods which can be employed in the decaffeination process. The least expensive is to use an organic solvent, such as dichloromethane, along with water to wash the beans, however there have been health concerns about residual levels of solvent in the coffee bean with this method and so it is no longer used in certain parts of the world, such as Europe and the United States (61).
water-soluble flavour molecules and chlorogenic acids (CGAs) are also lost in this process which can impact on the coffee flavour. Other methods use water or supercritical carbon dioxide (carbon dioxide held at high pressure and temperature >31 °C). The supercritical carbon dioxide method is more costly but has the advantage of being caffeine specific, thus retaining the flavour of the coffee. After decaffeination the beans are dried again, prior to roasting.

The temperature and duration of the roasting process can vary considerably depending on the type of roaster (drum or fluid bed) and the desired degree of roasting in the end product. Temperatures typically range from 180 – 250 °C, with roasting times as short as two minutes at higher temperatures, increasing up to 25 minutes for lower temperatures \(^{(64)}\). These extreme processes result in major changes to the chemical composition of the coffee beans.

### 1.3.3 Composition of green coffee beans

Of the many compounds present in green coffee beans, CHOs comprise the largest proportion, providing almost 60% of dry weight. Other components include lipids, proteins, polyphenols, caffeine and trigonelline, quantities of which vary between bean varieties, with Arabica beans containing smaller amounts of CHO, caffeine and polyphenols and larger amounts of sucrose, free amino acids and diterpenes than Robusta beans. The main components of green coffee are listed in Table 1.3.3-1.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Proportion (% dry weight)</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>carbohydrates</td>
<td>60</td>
<td>soluble and insoluble polysaccharides: cellulose, arabinogalactan, galactomannan oligosaccharides: stachyose, raffinose disaccharides: sucrose monosaccharides: glucose, galactose, arabinose, fructose, mannose, manitol, xylose, ribose</td>
</tr>
<tr>
<td>lipids</td>
<td>8 - 18</td>
<td>triglyceride (75%), sterols, fatty acids (e.g. linoleic, linolenic, stearic, oleic, palmitic), diterpenes (cafestol, kahweol)</td>
</tr>
<tr>
<td>proteins, peptides and amino acids polyphenols</td>
<td>9 - 16</td>
<td>asparagine, glutamic acid, alanine</td>
</tr>
<tr>
<td>polyphenols</td>
<td>6 - 10</td>
<td>CGAs (CQAs, FQAs, dCQAs), cinnamoylquinic acids, cinnamoyl-amino acid conjugates, cinnamoyl-glycosides</td>
</tr>
<tr>
<td>caffeine</td>
<td>0.9 - 2.5</td>
<td>0.9 - 1.3 % in Arabica, 1.5 - 2.5 % in Robusta beans</td>
</tr>
<tr>
<td>trigonelline</td>
<td>1</td>
<td>theobromine, theophylline</td>
</tr>
<tr>
<td>other compounds</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data from Ludwig et al., 2014 \(^{(64)}\). CGA: chlorogenic acid; CQA: caffeoylquinic acid; FQA: feruloylquinic acid.
1.3.4 Composition of roasted coffee beans

Coffee roasting, whilst being a relatively straightforward procedure, results in major changes to the chemical composition of the coffee beans. In the early stages of the roasting process free water evaporates then, as the coffee bean temperature increases, chemical reactions begin to take place. Sucrose begins to caramelise at about 130 °C, at which point the beans also begin to swell and turn brown. At higher temperatures, over 180 °C, pressures of up to 7 atm build up inside the coffee bean and many chemical transformations take place. One of the key reactions taking place during the roasting process is the Maillard reaction, which produces a variety of end products, including melanoidins which make up approximately 25% by weight of roasted coffee. Almost a thousand volatiles are produced during roasting, many of which are key to the aroma and taste of roast coffee.

Roasting results in a reduction in the amount of CHO, proteins, lipids and CGAs in the coffee bean, the extent of which is determined by roasting temperature and duration. Caffeine content, however, remains virtually unchanged. Most of the CGAs present in green coffee are lost during roasting, with up to 10% loss per 1% loss of dry matter. When one considers that 9 – 10% dry matter may be lost in a very dark roast (65), then this reduction in CGA content can be considerable, although such very severe roasts are not the norm. Nevertheless coffee is still the major source of CGAs in many people’s diets (66), with the main CGAs in coffee being caffeoylquinic acids (CQAs), feruloylquinic acids (FQAs), dicafeoylquinic acids (diCQAs), p-coumaroylquinic acids (pCoQAs) and caffeoylferuloylquinic acids (CFQAs) (67). Many transformations also occur to the CGAs during roasting, resulting in a multitude of end-products including cinnamic acids, quinic acid, quinides, caffeoylquinic lactones (CQL), alkyl esters and caffeic acid derivatives. Roasting also results in degradation of trigonelline to a variety of compounds including niacin (vitamin B3) and several volatiles. In addition to niacin, roast coffee also contains several other micronutrients including magnesium, potassium and vitamin E.

An analysis of six commercial roast ground coffees reported a caffeine range of 843 – 1456 mg / 100 g (lowest: medium roast, 100% Arabica; highest: light-medium roast Arabica/Robusta blend); and a trigonelline range of 280 – 956 mg / 100 g (lowest: dark roast, 100% Arabica; highest: light-medium roast, 100% Arabica) (68).

The main components of roasted coffee and their relative proportions are detailed in Table 1.3.4-1.
1.3.5 Effects of the decaffeination process on coffee composition

Decaffeination does not remove all caffeine from the coffee bean, but instead reduces the amount from 1 – 2 g / 100 g to 0.02 – 0.30 g / 100 g (69). UK legislation permits a maximum caffeine content of 0.1% dry weight for decaffeinated coffee beans and 0.3% dry weight for decaffeinated instant coffee (70).

As stated previously, the decaffeination process can alter the proportions of other key coffee components dependent on the decaffeination method. Decaffeination using organic solvents has been reported to produce CGA losses of 16% in Arabica beans and 11% in Robusta beans (71), with CGA losses also reported for water decaffeination methods (61).

1.3.6 Instant coffee composition

The composition of instant coffee is largely determined by the blend of beans used and roasting methods, as discussed previously. However the methods used to extract and concentrate the coffee also have an effect, with increased losses of thermolabile compounds, such as CGAs, occurring when extreme heat is used.

An analysis of four decaffeinated and five caffeinated instant coffees, reported total CGA content ranging from 43 – 55 mg for decaffeinated coffee (DC) and 37 – 77 mg for caffeinated coffee (CC) in a single serving (1.8 g instant granules made up in 200 ml water), with 5-CQA being the most abundant CGA in all coffees tested (72). Lipid content of instant coffee tends to be low and has been reported to range from 1.8 – 6.6 mg per 150 ml cup (73).

The caffeine content of instant CC has been reported to range from 2.5 – 5.0 g per 100 g (61), equivalent to 45 – 90 mg per 1.8 g serving. However, this “standard"
serving size of 1.8 g does not take into account individual preferences. Indeed, a Canadian study which analysed the caffeine content of various instant CCs prepared to participants' personal taste preferences, found a median caffeine concentration in the prepared coffees of 328 µg/mL (range 102 – 559 µg/ml) \(^\text{(74)}\). This median value would provide 66 mg caffeine in a 200 ml serving, which falls within the range reported for the standard serving size, but the extremes reported in the Canadian study (20 – 112 mg per 200 ml) do not, highlighting the dangers of relying on data from so-called “standard” measures when reporting on coffee intake. This is even more relevant when variation in serving size is also considered.

Nevertheless, as instant coffee is prepared simply by the addition of hot water to the instant coffee granules, variability between drinks for a particular batch of instant coffee is very low and determined only by the accuracy of measurement of the coffee granules and water volume. This makes instant coffee a good choice for intervention-type studies where standardisation and reproducibility of the test coffee is of high importance.

### 1.3.7 Composition of brewed coffees

Brewing method can have a significant impact on the composition of the end product. Factors such as particle size of the coffee grind, length of time in contact with water, water volume and temperature, filter material and, in the case of espresso coffees, water pressure, all have an impact on the final brew.

Use of a paper filter tends to remove most of the lipid fraction, resulting in brews containing less than 7 mg total lipid, compared to boiled coffees, which can contain up to 160 mg lipid per cup; those prepared with a metal filter, such as a mocha/stove-top pot, have been reported to contain intermediate amounts of up to 50 mg per cup \(^\text{(73)}\). Reductions in chlorogenic lactones and 5-CQA along with an increase in 3-CQA and 4-CQA have been observed when coffee is kept hot for a prolonged period, such as commonly occurs with filter coffee kept on a hot plate \(^\text{(75)}\).

An analysis of eight commercial ground roast coffees, brewed in a cafetiere for 4 min, reported total CGA content to range from ~27 mg (French blend; medium-dark roast; 40:60 Arabica:Robusta ratio) to ~94 mg (Breakfast blend; light roast; 100% Arabica) for a standardised serving (11 g roast coffee) \(^\text{(72)}\).

Amounts of the key coffee components vary considerably for espresso coffees, partly due to the complexity of the preparation method and partly due to variation in serving size. One analysis of espresso coffee, from 20 different commercial establishments in
Glasgow, reported a variation in serving size of 23 – 70 ml, with total CQA ranging from 24 – 422 mg and caffeine ranging from 51 – 322 mg per serving \(^{(76)}\). Another study purchased espresso from 26 coffee shops, in Scotland, Italy and Spain, and reported variations in mean serving size (15 – 83 ml), caffeine (48 – 317 mg) and total CQA (6 – 188 mg) \(^{(77)}\). They also observed noticeable differences between countries, with Italy favouring smaller servings of darker roasts, resulting in lower total CQA and Spain preferring larger servings of lighter roast coffees, with higher CQA content; the greatest variation was observed in the Scottish samples.

Illustrating the scale of the effect that the barista can have on the final coffee composition is one study which analysed six samples of Starbucks® Breakfast Blend coffee, purchased on consecutive days from the same coffee shop in Florida, and found a variation in caffeine content of 300 – 498 mg in a 16 oz. serving \(^{(78)}\).

Taking the above points into consideration, it is clear that, even following a standardised brewing method, there is greater opportunity for variation in the composition of the end product when brewed coffee is used in comparison to instant. This should be considered when choosing a coffee preparation method for trials investigating coffee’s effects, particularly if participants will be required to brew their own coffee. Furthermore, this variability should also be considered when interpreting epidemiological data.

### 1.3.8 Bioavailability of coffee components

Caffeine is absorbed rapidly in the small intestine, reaching peak plasma concentrations \((C_{\text{max}})\) within 30 – 60 min and with a half-life of around 5 h \(^{(79)}\), although this can vary considerably, with smokers exhibiting faster caffeine metabolism and pregnancy, oral contraceptives and liver disease all resulting in slower caffeine metabolism. Caffeine is metabolised in the liver, by the cytochrome P450 enzyme, CYP1A2, with the primary metabolites being paraxanthine, theobromine and theophylline. The plasma or salivary paraxanthine \((17X)\) to caffeine \((137X)\) ratio is frequently used as a marker of CYP1A2 activity \(^{(80)}\).

Coffee CGAs are extensively metabolised, with one study finding only trace amounts of 5-CQA and slightly higher amounts of FQAs appearing in the circulation following coffee consumption \(^{(67)}\). In contrast Monteiro et al. observed 3-, 4- and 5-CQA in the plasma of all of their participants with 4- and 5-FQA only present in one participant, leading them to conclude that there is large inter-individual variation in absorption and metabolism of CGAs in humans \(^{(81)}\). Some of the CQAs and FQAs are broken down in
the small intestine, releasing caffeic and ferulic acids which are then sulphated before absorption, however most of the ingested CGAs pass intact to the colon where they are extensively metabolised by bacterial enzymes prior to absorption (67). Analysis of ileal fluid from ileostomy patients indicates that almost 70% of ingested CGAs will pass from the small intestine to the colon (82). The main metabolites absorbed in the small intestine, and appearing in the circulation after approximately 1 h, are caffeic acid-3'-O-sulphate and ferulic acid-4'-O-sulphate, with DHCA, dihydroferulic acid (DHFA) and their sulphates being absorbed predominately in the colon and appearing in the circulation 4 - 6 h after ingestion (64). *In vitro* work has demonstrated gastric metabolism of several CGAs, resulting in the release of isoferulic acid, dimethoxycinnamic acid and ferulic acid with subsequent absorption across the gastric membrane (83). This may explain the rapid appearance of some metabolites, such as dimethoxycinnamic acid, which has been observed to reach peak levels in plasma after 30 min (84).

Trigonelline and *N*-methylpyridinium, a degradation product of trigonelline formed during the roasting process, are both absorbed intact, reaching $C_{\text{max}}$ within 3 and 2 h respectively; they are not extensively metabolised, with 46 - 57% of trigonelline and 69% of *N*-methylpyridinium being excreted in urine within 8 h (85). Studies in ileostomy patients found that approximately 70% of ingested cafestol and kahweol is absorbed in the small intestine, with less than 10% reaching the colon and a very small amount being excreted in urine, indicating extensive metabolism of these compounds (86).

### 1.4 Coffee and glucose metabolism

#### 1.4.1 Epidemiology

Epidemiological evidence for an inverse relationship between coffee consumption and T2DM risk is strong. It was first suggested in 2002 by van Dam *et al.*, who carried out a prospective cohort study in over 17,000 Dutch men and women between 1987 and 2000, and found that people who drank more than six cups of coffee per day were half as likely to develop T2DM as people who drank less than three cups per day (87). Many more papers were published in subsequent years, encompassing populations from the United States (5,88), Asia (2) and Europe (89,90). One study concluded that coffee and tea only conferred a benefit in those under 60 years old who had previously lost weight (88). Another, which examined data from nearly 70,000 French women who were part of the EPIC cohort, found that the association between coffee drinking and reduced T2DM
risk only applied when coffee was consumed at the lunchtime meal \( (91) \); they found no association between tea drinking and T2DM risk in this cohort.

A systematic review and meta-analysis in 2009 included 18 studies with a total of over 450,000 participants \( (92) \). They found a 7% reduction in relative risk (RR) of T2DM for every additional cup of CC consumed, with people drinking 3 - 4 cups/day having a 25% lower risk than those who drank 2 or fewer. Six of their included studies examined the effects of DC and when these were combined they found a 33% reduction in T2DM risk for those who drank 3 - 4 cups/day compared to those who drank none. Similarly, when the results of 7 studies investigating the effects of tea were combined, they found those who drank 3 – 4 cups/day had a 20% lower risk than those who drank none. They had insufficient data to determine a dose-response relationship in either tea or DC.

Two later meta-analyses \( (93,94) \), performed on 28 and 26 prospective studies respectively, found a dose-response reduction in RR of T2DM for CC, DC and caffeine; RR for 4 cups/day of any type of coffee was reported as 0.75 – 0.84 and for 6 cups/day was 0.67 – 0.78. Jiang et al. extended their analysis to 10 cups per day and reported a RR of 0.61 in this category \( (94) \). A recent analysis of data from the Nurses’ Health Study found a 17% increased risk of T2DM in people who had reduced their coffee intake by more than 1 cup/day over a four year period along with an 11% reduction in risk for those who had increased consumption by more than 1 cup/day \( (95) \).

Other cohort studies have investigated links between coffee drinking and other markers associated with T2DM risk. Five or more cups of coffee per day was associated with lower incidence of impaired glucose tolerance (IGT) \( (96) \) and lower fasted insulin, but not fasted glucose \( (97) \). One study examined various components of MS and found that coffee consumption was associated with lower HDL cholesterol in women but not men; however they found no associations between coffee and fasted glucose, TAG, waist circumference or blood pressure (BP) in either men or women \( (98) \).

A recent cross-sectional study in a Korean population found an increased risk of abdominal obesity, low HDL cholesterol and MS in those who drank more than three coffees per day \( (99) \). The authors hypothesised that this may be due to the type of coffee consumed by the participants, as most of them drank an instant coffee mix containing sugar and creamer, and this additional sugar and fat in the diet may have been contributing to the apparent effect of coffee. Conversely, a Japanese study found significant inverse associations between coffee drinking and all components of MS.
apart from HDL cholesterol \(^{(100)}\). Another recent study found no association between either Turkish coffee or instant coffee and fasted lipids \(^{(101)}\).

Coffee consumption has been associated with higher adiponectin levels in both women \(^{(102)}\) and men \(^{(103)}\), but others have found no such association \(^{(104)}\). Conflicting results have also been found with respect to coffee drinking and inflammatory markers; the ATTICA study found coffee drinking to be associated with increased levels of IL-6 and TNF-\(\alpha\) \(^{(105)}\), whereas others have found no association with IL-6 \(^{(102)}\) and lower levels of TNF-\(\alpha\) in coffee drinkers \(^{(106)}\).

Whilst the epidemiology puts a strong case for coffee drinking having a protective effect in terms of reduced risk of development of T2DM, caution is advisable in interpretation of the evidence relating to a dose-response relationship. As discussed previously, variation in coffee beans, degree of roasting, preparation method and serving sizes mean that there is no such thing as a typical cup of coffee. Indeed, an individual consuming 10 cups/day might conceivably receive less CGAs and caffeine than an individual consuming only one cup per day. While this extreme scenario is unlikely to occur very often, a four- to five-fold variation is potentially commonplace. When one also considers that much epidemiological data is gathered retrospectively, relying on an individual’s recall of their intake over several years, then the accuracy of any RR for a particular number of cups of coffee is questionable.

### 1.4.2 Acute effects of coffee on glucose metabolism

In contrast to the epidemiology, many, but not all, acute studies have demonstrated a detrimental effect of coffee on glucose metabolism.

An early study by Acheson et al. compared the effects of CC, containing 4 mg caffeine/kg bodyweight (BW), with DC on fasted glucose and insulin in an obese group \((n=6)\) over a 3 h period and found no effect of CC or DC on either glucose or insulin over that time period \(^{(107)}\).

Another early study by Feinberg et al. examined the acute effects of CC on glucose tolerance in 23 healthy participants and found that CC reduced blood glucose at 30 and 60 min after consumption compared to placebo \(^{(108)}\). They also demonstrated higher serum NEFA at 180 min following CC consumption and no effect on serum insulin at any time-point. They hypothesised that the effect on NEFA at the 3 h mark was solely due to caffeine as the suppressive effect of insulin on NEFA would have dissipated by then. Unlike later studies, they varied the glucose dose administered to their participants by bodyweight (giving 1 g glucose / kg BW), whilst keeping the CC
dose constant (5 g instant CC, containing 220 mg caffeine). They also did not calculate area under curve (AUC) or incremental area under curve (IAUC), making comparisons with later studies difficult.

In contrast to Feinberg et al., many subsequent studies have observed an increase in postprandial glucose following CC ingestion compared to a control (7,109,110). Battram et al. compared caffeine with CC, DC and control (10). Their caffeine and CC both provided 4.45 mg /kg caffeine. They administered their test drinks 60 min prior to a standard oral glucose tolerance test (OGTT) and found caffeine increased glucose and insulin AUC more than CC compared to both control and DC. They also observed less of an increase in glucose following DC than following the control. They also found caffeine increased NEFA and glycerol in the 60 min prior to the OGTT, whereas CC did not, despite CC and caffeine producing similar levels of adrenalin in the same time period. This led them to hypothesise that other components in coffee were attenuating the effects of caffeine on both glucose disposal and lipolysis. The effects of individual coffee components will be discussed further, later in this chapter.

A systematic review of all studies to date which have investigated the acute effects of coffee on postprandial glucose metabolism can be found in Chapter 3 of this thesis.

1.4.3 Longer-term effects

There have been few longer-term intervention studies into the effects of coffee on glucose metabolism, the majority of which have examined the effects in regular coffee drinkers (11–13).

Van Dam, Pasman and Verhoef carried out two trials in coffee drinkers (12). In the first, they got participants to drink a litre of filtered CC (made from 70 g of coffee grounds) per day for 4 weeks and compared this to 4 weeks of coffee abstention in a cross-over design. Despite their participants regularly consuming more than 5 cups/day prior to the trial, they still had a large number of dropouts (10/40) due to side effects from the coffee. This is probably due to the very high dose administered or the strength of the drink. The authors reported their dose to be the equivalent of 13 cups of coffee in one litre of liquid. Despite this very high dose they found only a transient effect on fasted glucose: CC tended to increase glucose at 2 weeks (p=0.08) but not after 4 weeks, perhaps indicating a degree of acclimatisation. They did however find CC increased fasted insulin after 4 weeks. Their second study, reported in the same paper, also utilised a crossover design and compared 2 weeks each of filtered CC (52 g in 0.9 l), caffeine (870 mg in six capsules) and placebo (6 cellulose capsules). This study had
fewer dropouts for coffee-related issues (3/54). The caffeine content of the filter CC was not reported so it is unknown whether the CC and caffeine treatments were comparable for caffeine, although 870 mg caffeine in 52 g CC, equating to 1.7% caffeine, would not be unreasonable. In this study they found no effect of either caffeine or CC on fasted glucose, but reported a tendency for higher insulin after both CC and caffeine ($p=0.15$). They did not report any washout period for either study, either before the studies began or between treatment phases.

In a non-crossover design, Kempf et al. recruited regular coffee drinkers who had an elevated risk of T2DM (11). They were asked to drink no coffee for four weeks, followed by 4 cups of CC/day for another four weeks then 8 cups of CC/day for a final four weeks. Most of their participants were female and obese and had a mean coffee consumption pre-trial of 4 cups/day. They found no effect of CC on either fasted glucose or insulin, nor did they find an effect on 30 min and 2 h glucose and insulin after an OGTT. They did however report that their lower dose of 4 cups/day increased HDL cholesterol and their higher dose of 8 cups/day increased both total and HDL cholesterol with no effect on LDL cholesterol or TAG versus coffee abstention. They also observed a decrease in IL-18, with no effect on IL-6 or CRP. Their lower dose of 4 cups/day was the same as the mean pre-intervention intake of the participants, so it is perhaps surprising that they observed an effect on HDL cholesterol from that dose. However, their participants underwent a four week caffeine washout prior to the trial, which may be a sufficiently long period of time to reverse any effects of habitual coffee consumption on HDL cholesterol. Unfortunately they did not take any baseline measures before the washout period, so it is not possible to determine whether there was any effect from the washout period.

In a parallel-arm trial, Wedick et al. gave their participants (n=45, across three groups) either 5 cups/day instant CC, 5 cups/day instant DC or control (no coffee) for 8 weeks (13). Their participants were overweight (mean BMI = 29.5 kg/m²) regular coffee drinkers (2 or more cups/day). All groups had a 2 week caffeine-free washout period prior to their first visit. Coffees were provided in 2 g portions and participants were instructed to make it up with 177 ml boiling water. This resulted in a reported daily dose of 345 mg caffeine, 302 mg CGA and 78 mg trigonelline in the CC and 216 mg CGA and 65 mg trigonelline in the DC. There were no differences between control and either CC or DC for glucose AUC, 2 h glucose or insulin, nor was there any effect on HDL, LDL or TAG. CC increased adiponectin versus control.
Kempf et al. carried out another longer-term intervention in coffee drinkers, where they compared the effects of a medium roast (MR) and a dark roast (DR) CC in 114 overweight individuals (111). In a parallel-arm study, with no control group, their participants were asked to drink at least 3 cups per day of their allotted CC over 12 weeks, after an initial 4 week washout period. They observed a small increase of 0.1% in HbA1c values for both groups. In the MR group they saw an increase in HDL cholesterol and adiponectin, whereas they saw an increase in TAG and a decrease in systolic blood pressure (SBP) in the DR group. These changes did not remain significant after correction for multiple tests. They found no effect of either coffee on weight, BMI, waist circumference, diastolic blood pressure (DBP), fasted glucose, fasted insulin, HOMA-IR, total and LDL cholesterol.

One study recruited people who were not heavy coffee drinkers (mean consumption: 2 cups/week of coffee and 5 cups/week of tea) and found a reduction in the postprandial glycaemic response following a 16 week coffee intervention (112). It was a parallel-arm design with a total of 43 participants randomised to one of three treatments: 5 cups/day of instant CC, instant DC or water for 16 weeks. Their participants were overweight Japanese men, aged 40 - 64 y, with elevated fasted glucose. They observed a decrease in AUC glucose and 2 h glucose in both CC and DC groups compared to control, however the results were only statistically significant for the DC group after adjustment for change in waist circumference. They observed no change in insulin in any group.

**Effects on blood lipids**

In a randomised cross-over study, 20 healthy, habitual coffee drinkers drank 3 - 4 cups of medium roast (MR) or medium light roast (MLR) filtered CC per day for four weeks (113). Total and LDL cholesterol increased significantly in each group with no difference between groups. HDL cholesterol increased significantly more with MR than with MLR. They observed no effect on TAG, IL-6 or TNF-α. The authors noted that their coffees had higher amounts of cafestol and kahweol than is usually found in paper filtered coffee and hypothesised that this may be the reason for the increase in lipids in their study as these two compounds have been previously identified as lipid-raising (114,115). Another randomised parallel-arm trial, over 8 weeks, also found filtered CC raised total, LDL and HDL cholesterol compared to a control (116), although they found the effect only in their highest dose group (720 ml/day), with no effect in a lower dose (360 ml/day) nor in a DC group (720 ml/day).
This is in contrast to an earlier parallel-arm study which found boiled CC raised total and LDL cholesterol compared to filter CC, with no effect of filter CC compared to a control (117). However, their participants were instructed to drink 3 cups/day of filter CC in the run-in phase, with the active phases being 4-6 cups/day of either boiled CC, filter CC or no coffee for 9 weeks. It is possible that the increase from 3 to 4 - 6 cups/day in the filter CC group was insufficient to produce a noticeable difference in results over this time-frame.

Two meta-analyses have been performed on the effects of coffee on serum lipids, with both finding a dose-response effect of coffee on total and LDL cholesterol and TAG (118,119). Both found the effect to be stronger in dyslipidaemic patients and in trials using caffeinated and boiled coffees.

1.4.4 Potential mechanisms

Several mechanisms have been postulated to explain the apparent protective effects of coffee on T2DM risk suggested by the epidemiology. These include aiding weight loss by increasing energy expenditure and fat oxidation (120,121) and reducing energy intake (122). Caffeinated coffee has been demonstrated to reduce energy intake at a subsequent meal and over the course of a whole day in overweight but not in normal-weight individuals, without any noticeable difference in feelings of hunger or satiety (122). However, the CC used in this study provided a high dose of caffeine (6 mg/kg BW) and it is unclear whether normal coffee drinking would produce the same effect or whether these acute effects would persist over a longer time frame. One study compared the effects of a DR with a light roast (LR) CC and found that the DR significantly reduced bodyweight in overweight participants by 2.5 kg over four weeks, but not in normal-weight, whereas the LR resulted in a slight increase of 0.2 kg (123). The weight loss can be ascribed to the observed reduced energy intake in the DR group. It is unlikely that this rate of weight loss would be sustainable over several months and indeed, another trial comparing DR and medium roast (MR) CCs did not see any difference in bodyweight for either CC over a three month period (111).

Some studies have demonstrated an increase in circulating adiponectin levels following coffee consumption (13,112). Adiponectin levels have been reported to be inversely associated with insulin resistance (124), so an increase in levels may result in improved insulin sensitivity, although this has not been observed previously perhaps due to short study durations.
Some of the effects of specific coffee components are discussed further in the following subsections.

1.4.4.1 Effects of caffeine

Greater glucose and insulin AUCs have been demonstrated following caffeine ingestion compared to both control (125) and DC (126), with the effect being stronger than that produced by CC (10). Caffeine has been observed to raise plasma adrenalin and to act as an antagonist to both adrenergic and adenosine receptors, all of which may contribute to its observed glucose raising effects. However it has been suggested that the acute adrenalin-raising effects of caffeine are temporary, as chronic caffeine exposure of just seven days has resulted in acclimatisation to its effects (127).

Acute caffeine ingestion is known to decrease glucose disposal in both sedentary and exercise trained individuals (128,129). It has been suggested that this inhibition of glucose disposal is solely a result of increased adrenalin as the effects were negated when propanalol, a beta-adrenergic antagonist was administered along with caffeine (69). A later study, however, found a difference in the reduction in glucose infusion rate (GIR) during an euglycaemic-hyperinsulinaemic clamp between a low dose adrenalin infusion and an oral caffeine dose, with the caffeine showing a significant reduction in GIR compared to adrenalin infusion, despite the oral caffeine producing a similar, but lower, adrenalin concentration as the infusion (130). This led them to conclude that increased adrenalin following caffeine consumption was not the only mechanism for the subsequent reduction in glucose disposal. They did not find any difference between treatments in endogenous glucose production or NEFA, leading them to postulate that their effects may be due to adenosine receptor antagonism. Adenosine is an endogenous nucleoside which binds to a variety of receptors and regulates insulin secretion, glucose uptake and release, and is involved in adipose tissue lipolysis. It can have both stimulatory and inhibitory effects on glucose metabolism depending on which receptors it binds to (131). As caffeine is a non-specific adenosine receptor antagonist (132), this is a plausible contributory mechanism to the observed reduction in glucose disposal following caffeine ingestion.

Caffeine has also been demonstrated to inhibit cyclic nucleotide phosphodiesterase which is involved in the breakdown of cAMP (133) and appears to increase cAMP concentrations above levels observed with adrenalin administration alone (134). As increased concentrations of cAMP lead to increased blood glucose, as discussed in Section 1.2.4, this is another possible mechanism by which caffeine may increase the postprandial glucose response.
Acheson et al. compared caffeine (8 mg/kg BW) with placebo (0.5 g glucose) and took blood samples at regular intervals for 3 h after ingestion of the respective treatments. They found that caffeine increased metabolic rate by 16%, but with high inter-individual variation (107). They observed no effect on glucose or insulin but did report an increase in NEFA in both treatments, with caffeine increasing NEFA more than placebo. The increase in NEFA following caffeine ingestion is in direct contrast to another study reported in the same paper, where they gave CC or DC to an obese group of participants and observed no effect on NEFA over the same time period, however this lack of an effect of coffee on NEFA in obese participants has been reported by others (135) and has been suggested to be a result of reduced responsiveness to lipolytic stimuli in the obese.

Caffeine may have a beneficial effect as an antioxidant. In vitro work using physiological concentrations demonstrated no effect of caffeine or its primary metabolites, theophylline, paraxanthine and theobromine, on LDL peroxidation, but did observe a protective effect from the theophylline and paraxanthine derivatives, 1-methylxanthine and 1-methyluric acid (136).

1.4.4.2 Effects of chlorogenic acids

Glucose metabolism

It has been suggested that bioactive components in coffee, other than caffeine, may be attenuating the documented acute negative effects of caffeine on glycaemia, with CGAs and their metabolites attracting attention as possible candidates. Some suggested mechanisms include delayed digestion of starches, delayed glucose absorption and increased glucose uptake from the circulation.

Chlorogenic acids may delay starch digestion, as in vitro work has demonstrated partial inhibition of pancreatic α-amylase by both 5-CQA and caffeic acid (137) and inhibition of α-glucosidase by 3,5-diCQA (138).

Increased disposal of glucose in non-skeletal muscle has been demonstrated in animal models following a gastric infusion of a synthetic quiniide, however the concentration administered was higher than would be achieved through normal coffee drinking (139).

In vitro work has demonstrated an 80% reduction in glucose uptake by the enterocyte when treated with 5-CQA and 30 – 40% reduction when treated with ferulic and caffeic acids, with dissipation of the sodium gradient and consequent inhibition of the SGLT1 glucose transporter being suggested as a potential mechanism (140). Under normal physiological conditions however, if glucose concentrations in the gut became high,
the facilitative transporter, GLUT2, would translocate to the brush border membrane and take over glucose transport, thus minimising any effect of SGLT1 blockade. Reduced glucose transport via GLUT2 has been observed following incubation with a herbal supplement containing a mixture of polyphenols some of which were derived from coffee beans, however the supplement contained a range of herbs and spices and it is unknown which particular components were responsible for the observed effect \(^{(141)}\).

Chlorogenic acids may also reduce hepatic glucose output, as \emph{in vitro} \(^{(142)}\) and animal \(^{(143)}\) models have demonstrated inhibition of G6Pase by CGAs.

It would appear from the above that there is potential for a beneficial effect of CGAs in terms of reducing the postprandial glycaemic response. Giving weight to this hypothesis, studies involving CGA on its own \(^{(144)}\), as green coffee extract (GCE) \(^{(145)}\) and coffee enriched with CGA \(^{(146)}\) have demonstrated a reduction in glucose response \emph{versus} control. It should be remembered, however, that these studies have used high doses of CGA, certainly higher than that delivered by a typical beverage; the majority of studies using regular coffee, both CC and DC, have failed to find any beneficial effect on the acute glucose response.

Johnston \emph{et al.} controlled the amount of total CQA in their coffees when comparing the effects of CC and DC on the postprandial response; both their CC and DC provided a CQA concentration of 2.5 mmol/L \(^{(147)}\). The authors observed lower plasma GIP concentrations following consumption of both coffees compared to control, particularly with DC in the first 30 min. They also reported higher plasma GLP-1 concentrations following DC than control over the first 120 min. This led them to conclude that the altered patterns of GIP and GLP-1 were a result of delayed glucose absorption in the small intestine as a result of the actions of coffee components, possibly CGAs. It should be noted however that they found no overall effect of either CC or DC on 3 h glucose and insulin response, raising the question of whether the observed delay in glucose absorption is physiologically relevant. Furthermore, the doses of coffee given (12 g DC and 8.7 g CC) are not typical of normal coffee consumption, as a typical serving of instant coffee is 2 g. It is not known whether they would have observed the same effects at a lower dose.

\textit{Weight loss}

A meta-analysis of studies investigating the effects of GCE on weight loss was carried out in 2011, with three trials selected for inclusion \(^{(148)}\). The authors reported that GCE significantly reduced bodyweight, with a mean reduction of 2.5 kg, but noted that the
included trials were very heterogeneous, poorly carried out, included low numbers of participants (n = 30–62) and were of short duration (4–12 weeks). Doses of GCE given ranged from 180 to 200 mg. Longer and more rigorously controlled trials are required before any conclusions can be drawn regarding the efficacy of GCE as a weight loss treatment.

Antioxidant capacity

Whilst CGAs may exhibit antioxidant activity in vitro, the concentrations attained in vivo are low, as discussed in Section 1.3.8, and their ability to make a significant contribution to overall antioxidant activity has now been called into question (149).

1.4.4.3 Effects of trigonelline and niacin

Trigonelline may have some beneficial effect on the glycaemic response. One study observed lower glucose and insulin at the 15 min time-point during an OGTT following trigonelline ingestion in comparison to a placebo (144). However, the dose given (500 mg) was substantially higher than would normally be present in coffee and they did not find an overall effect, so any beneficial effect from trigonelline in the concentrations found in coffee is likely to be small.

The effects of caffeine on NEFA may be partially offset by niacin, which has been demonstrated to reduce NEFA and is prescribed as a drug to treat hyperlipidaemia, although in the long term it has a rebound effect and leads to elevated plasma NEFA (150).

1.4.4.4 Effects of cafestol and kahweol

The diterpenes cafestol and kahweol are generally thought to have a detrimental effect on health as they have been demonstrated to increase serum cholesterol and TAG (114,115), although it has been suggested that cafestol is responsible for most of the effect (151). Recently however, an in vitro study has demonstrated increased insulin secretion from pancreatic beta cells and increased glucose disposal into skeletal muscle cells following incubation with cafestol, suggesting a potential beneficial effect of this compound (152).

1.4.5 Effect of genotype

A gene is a segment of DNA which encodes a specific genetic function. A single nucleotide polymorphism (SNP) is a variation in one nucleotide at a particular position on the genome, which is present in at least 1% of the population (153). If the SNP occurs
within a gene it results in different versions of that gene, known as alleles. An individual has two copies of each gene, which together form that person’s genotype. The phenotype is the physical manifestation of the genotype. If only one copy of a particular allele is required to produce a specific phenotype, that allele is classified as the dominant allele, whereas if two copies are required, the allele is classified as recessive (154).

The CYP1A2 gene encodes the P450-1A2 enzyme, the primary enzyme responsible for caffeine metabolism by the liver. A particular SNP in this gene, rs762551, affects caffeine metabolism, with carriers of the dominant, C allele considered to be slow caffeine metabolisers and those homozygous for the recessive, A allele considered fast metabolisers (155). For some genes, specific environmental conditions are required before expression of a particular phenotype. It is thought that the fast metaboliser phenotype requires induction by, for example, smoking (156,157) or heavy coffee consumption (158). However, for convenience in this thesis and in order to easily distinguish between the groups, those with the A/A genotype will be referred to as being of a fast metaboliser phenotype and those with the A/C and C/C genotypes as being of a slow metaboliser phenotype.

An association has been observed between phenotype and T2DM risk, with the slow phenotype appearing to confer an increased risk of T2DM irrespective of coffee consumption (159). Others have reported an association between coffee drinking and impaired fasted glucose (IFG) in those with the slow phenotype only (160). It is possible that the different phenotypes will respond differently to a coffee intervention, however to our knowledge this has not previously been explored.
1.5 Aims of current work

There are several gaps in the literature relating to coffee and glucose metabolism. The contradictory results from previous acute coffee studies may be partially explained by variation in coffee composition and the different effects of the individual coffee components as discussed previously, however this cannot be verified as the majority of these studies did not report a full coffee analysis. A further limitation to previously published work is the relatively high dose of caffeine found in the coffees, typically 3 - 6 mg caffeine/kg BW \(^{(126,161)}\), which equates to 210 - 420 mg for a 70 kg person, or the equivalent of approximately 3 – 6 servings of instant coffee taken as a single dose.

Additionally, there have been few longer-term interventions, the majority of which have been carried out in habitual coffee drinkers. It is likely that regular coffee drinkers will already have gained any potential longer-term benefits of coffee drinking and may therefore not be the most appropriate population to study.

The aims of the current work were, therefore, to attempt to fill some of the gaps in the knowledge base. Namely:

- To perform a full review of the literature relating to the acute effects of coffee on postprandial glycaemia (Chapter 3).
- To investigate the effects of "normal" doses of coffee, both CC and DC, on postprandial glycaemia (Chapters 4 and 5).
- To carry out a longer-term intervention in coffee-naïve individuals and to further examine any differences in response between slow and fast caffeine metaboliser phenotypes (Chapters 6 and 7).

1.6 Hypotheses

- A "normal" dose of CC will acutely increase the postprandial glycaemic response more than a control.
- A "normal" dose of DC will have no acute effect on postprandial glycaemia.
- Longer-term CC drinking will reduce the postprandial glycaemic response.
- Slow and fast caffeine metaboliser phenotypes will respond differently to longer-term CC drinking.
Chapter 2. General methods

This section describes in detail the methods followed for each of the studies in chapters 4 through 7 of this thesis. Specific study design will be included in each chapter.

2.1 Recruitment

All participants were recruited from the University of Surrey staff and students by email and poster advertisements and from the general public by word of mouth. All studies received a favourable ethical opinion from the University of Surrey Ethics Committee, details of which are provided in each chapter.

2.2 Screening

Respondents who expressed an interest in taking part in each study were invited to attend a screening session at the Clinical Investigation Unit (CIU) at the University of Surrey. During these sessions potential participants were given a full explanation of the study and had the opportunity to ask questions. If they then wished to proceed they were asked to sign study consent forms (sample forms can be found in Appendix 1). Following this they completed a brief health and lifestyle questionnaire (Appendix 2) and a habitual coffee and caffeine intake questionnaire (Appendix 3) to assess their suitability for inclusion in the study. Height and weight were measured and BMI calculated. Bioimpedance was used to measure body composition. A small amount of blood was collected by finger prick, from which fasted blood glucose and haemoglobin levels were measured. Those with fasted glucose $\geq 7.0$ mmol/L $^{(1)}$ and/or haemoglobin $< 13$ g/dl (men) / $< 12$ g/dl (women) $^{(162)}$ were excluded from the studies.

2.2.1 Anthropometry

Height was measured to the nearest half centimetre using a Harpenden stadiometer. All measurements were taken in bare feet with the participants standing with their backs to the stadiometer and looking straight forward.

Body composition was measured after an overnight fast and immediately after the participants had emptied their bladders. Different Tanita body composition analysers were used for each study. The studies which were carried out at the CIU (detailed in Chapters 4 and 5) used a Tanita MC-180MA whereas the final study (Chapters 6 and 7) carried out at the Surrey Clinical Research Centre (SCRC) used a Tanita BC
418MA. Both analysers work by passing a small electrical current through the body and measuring the resistance to that current. Bioimpedance analysis underestimates fat mass compared to the gold standard dual-energy X-ray absorptiometry (DXA) method \(^{(163)}\), however it is a cheap and non-invasive method which demonstrates a good correlation with DXA when measuring changes in body composition \(^{(164)}\). This underestimation of fat mass was not an issue for our studies as we were interested in relative change.

Blood pressure was measured using an Omron portable electronic BP monitor (Omron Healthcare UK Ltd, Milton Keynes, UK). It was measured in the seated position after the participants had been resting for five minutes. Two measures were taken, one minute apart, and the mean value calculated.

### 2.2.2 Screening blood samples

Fasted capillary blood samples were taken at the screening session to check that potential participants had fasted glucose and haemoglobin within the normal healthy ranges. A single-use safety lancet (Owen Mumford, Oxford, UK) was used to create a small puncture wound in the fingertip and the finger gently squeezed to produce a drop of blood. This blood was collected into HemoCue Glucose 201 and Hb 201 microcuvettes which were immediately inserted into the appropriate analyser (HemoCue Glucose 201 Plus analyser for measurement of glucose and HemoCue Hb 201 Plus analyser for measurement of haemoglobin (HemoCue Ltd, Sheffield, UK)).

### 2.2.3 Normal caffeine intake

Participants’ normal coffee and caffeine intake prior to taking part in the studies was assessed by questionnaire (Appendix 3). Estimates of their normal caffeine intake were made based on the following assumptions:

- single espresso: 140 mg caffeine \(^{(76)}\)
- cup of instant coffee: 54 mg caffeine \(^{(165)}\)
- cup of tea: 40 mg caffeine \(^{(165)}\)
- cola: 9.7 mg caffeine per 100 g \(^{(166)}\)
- small hot chocolate: 20 mg caffeine \(^{(167)}\)

All caffeine estimates should however be treated with caution, due to the large variation in caffeine content depending on source, preparation method and portion size as described previously in Chapter 1.
2.3 Blood sampling during postprandial tests

2.3.1 Capillary blood

Capillary blood samples were collected for the studies detailed in Chapters 4 and 5. After washing their hands, participants created a small puncture wound in their fingertip with a Unistik 3 Extra single-use safety lancet (Owen Mumford, Oxford, UK) and squeezed approximately 300 µl blood from the fingertip into a heparin fluoride microvette tube (Sarstedt, Leicester, UK). The tubes were immediately refrigerated at 4 °C until the end of the study session when they were centrifuged for 10 min at 1370 x g, 4 °C (Sigma 3-16PK) following which the plasma was transferred to serum microvette tubes (Sarstedt, Leicester, UK). These plasma samples were then frozen at -20 °C for batch analysis at the end of the study.

2.3.2 Venous blood

Venous blood samples were collected for the twelve week intervention detailed in Chapter 6. A cannula was inserted into the antecubital vein of each participant and remained in situ until the end of each study session. Cannulae were kept patent with saline solution. Blood samples were drawn from the cannula by a trained phlebotomist at regular time points throughout the session. At each time point a small amount of blood (approx. 1 ml) was drawn into a syringe and discarded to clear the cannula of any residual saline solution, then a further 2 ml was drawn into a syringe and transferred to a 2 ml fluoride oxalate tube (Teklab, UK) which was stored on ice until the end of the session. A further 4 / 8 ml was drawn into Vacuette® Z serum clot activator tubes (Greiner Bio-One, UK) which were kept at room temperature for 30 min to encourage clotting before being stored on ice until the end of the session. At the end of the study session the tubes were centrifuged for 10 min at 1912 x g, 4 °C (Legend™ T/RT Quikset centrifuge, Thermo Scientific, UK) following which the plasma and serum were transferred to 0.5 ml Apex plus microtubes (Alpha Laboratories, UK) and frozen at -20 °C until the end of the study.
2.4 Plasma and serum analysis

2.4.1 Analysis of plasma glucose

Plasma glucose was measured in two different ways, due to availability of equipment, on either the YSI 2300 Stat Plus analyser or the ILab 650 analyser. The method was however consistent for each study.

**YSI 2300 Stat Plus**

Plasma glucose for the dose-response (Chapter 4) and repeated-dose (Chapter 5) studies were measured on the YSI 2300 STAT Plus™ (YSI Life Sciences, UK). Samples were measured at the end of each study day. The YSI 2300 utilises a turntable that can hold 24 samples per run; this allows for a maximum of 18 participant samples plus two quality controls (QCs) and one standard to be measured at the start and end of each run. In addition, the YSI was set to auto-calibrate after every five samples. All samples for an individual participant for that day were measured on the same run to minimise inter-assay variation; this meant that a maximum of 2-3 participants were measured per run. Inter-assay CVs were ≤3% and intra-assay CVs were <2%. After being measured, samples were frozen and stored at -20 °C for future insulin analysis.

The YSI uses the glucose oxidase method to determine glucose concentrations. The glucose oxidase enzyme is immobilised in a membrane between layers of polycarbonate and cellulose acetate. When the blood sample passes through the membrane the glucose in it is oxidised by the enzyme, producing hydrogen peroxide ($\text{H}_2\text{O}_2$), which is then oxidised at a platinum electrode. The current produced is proportional to the glucose concentration of the sample. The chemical reactions are as follows.

\[
\text{Membrane reaction:} \quad \text{D-glucose} + \text{O}_2 \xrightarrow{\text{glucose oxidase}} \text{D-glucono-δ-lactone} + \text{H}_2\text{O}_2
\]

\[
\text{Anode reaction:} \quad \text{H}_2\text{O}_2 \xrightarrow{\text{platinum anode}} 2\text{H}^+ + \text{O}_2 + 2\text{e}^-
\]
ILab 650

Plasma glucose for the twelve week intervention (Chapters 6 and 7) was measured on the ILab 650 analyser (Instrumentation Laboratory, UK), using an IL Test Glucose (Oxidase) kit (Instrumentation Laboratory, UK). Two QCs were used, Human Control Assay 2 and 3 (Randox, UK) and the calibrant was IL Test ReferrIL G (Instrumentation Laboratory, UK). Up to 56 samples were placed on the carousel and analysed in batch. QCs were measured in triplicate at the start and end of each run. All plasma samples were measured in duplicate and the mean value calculated. All samples for one participant were analysed within the same batch. Inter-assay CVs were 2% for both low and high QCs and intra-assay CVs were ≤ 4% for both.

The ILab uses an enzymatic colorimetric method to measure glucose. Glucose in the sample is oxidised to gluconic acid and hydrogen peroxide. The hydrogen peroxide reacts with phenol and 4-aminoantipyrine to produce a red quinoneimine dye. The concentration of the dye is determined by measuring the absorbance at 510 nm and is directly proportional to the concentration of glucose in the sample. The chemical reactions are as follows:

\[
\beta-D\text{-glucose} + O_2 + H_2O \xrightarrow{\text{glucose oxidase}} \text{gluconic acid} + H_2O_2
\]

\[
2H_2O_2 + \text{phenol} + 4\text{-aminoantipyrine} \xrightarrow{\text{peroxidase}} \text{red quinoneimine dye} + 4H_2O
\]

2.4.2 Analysis of serum total cholesterol

Serum cholesterol was measured on the ILab 650 analyser (Instrumentation Laboratory, UK), using an IL Test Cholesterol kit (Instrumentation Laboratory, UK). Two QCs were used, Human Control Assay 2 and 3 (Randox, UK) and the calibrant was IL Test ReferrIL G (Instrumentation Laboratory, UK). Up to 56 samples were placed on the carousel and analysed in batch. QCs were measured in triplicate at the start and end of each run. All plasma samples were measured in duplicate and the mean value calculated. All samples for one participant were analysed within the same batch. Inter-assay CVs were 4% for both low and high QCs and intra-assay CVs were ≤ 9% and ≤ 3% respectively.
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The ILab uses an enzymatic colorimetric method to measure total cholesterol. Cholesterol ester is hydrolysed to free cholesterol, which is subsequently oxidised to produce \( \text{H}_2\text{O}_2 \). The \( \text{H}_2\text{O}_2 \) generated reacts with phenol and 4-aminoantipyrine to produce a red quinoneimine dye. The concentration of the dye is determined by measuring the absorbance at 510 nm and is directly proportional to the concentration of cholesterol in the sample. The chemical reactions are as follows:

\[
\text{cholesterol ester} + \text{H}_2\text{O} \xrightarrow{\text{cholesterol esterase}} \text{cholesterol} + \text{FFA}
\]

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

\[
2\text{H}_2\text{O}_2 + \text{phenol} + 4\text{-aminoantipyrine} \xrightarrow{\text{peroxidase}} \text{red quinoneimine dye} + 4\text{H}_2\text{O}
\]

### 2.4.3 Analysis of serum HDL-cholesterol

Serum HDL cholesterol was measured on the ILab 650 analyser (Instrumentation Laboratory, UK), using an IL Test HDL-Cholesterol kit (Instrumentation Laboratory, UK). Two QCs were used, SeraChem Control Level 1 and 2 (Instrumentation Laboratory, UK) and the calibrant was IL Test ReferrIL G (Instrumentation Laboratory, UK). QCs were measured in triplicate at the start and end of each run. All plasma samples were measured in duplicate and the mean value calculated. All samples for one participant were analysed within the same batch. Inter-assay CVs were 9% and 12% for the low and high QCs, respectively and intra-assay CVs were \( \leq 2\% \) and \( \leq 9\% \) respectively.

The ILab uses a two-step enzymatic colorimetric method to measure HDL cholesterol. Anti-human \( \beta \)-lipoprotein antibody binds to lipoproteins (LDL, VLDL and chylomicrons) other than HDL, blocking reaction of these lipoproteins with cholesterol esterase and cholesterol oxidase, which then react only with HDL cholesterol. Thereafter the method and reactions involved are the same as for total cholesterol, with a blue coloured dye formed from the final reaction with hydrogen peroxide. The concentration of the dye is determined by measuring the absorbance at 600 nm and is directly proportional to the concentration of HDL cholesterol in the sample.
2.4.4 Analysis of serum TAG

Serum TAG was measured on the ILab 650 analyser (Instrumentation Laboratory, UK), using an IL Test Cholesterol kit (Instrumentation Laboratory, UK). Two QCs were used, Human Control Assay 2 and 3 (Randox, UK) and the calibrant was IL Test ReferrIL G (Instrumentation Laboratory, UK). QCs were measured in triplicate at the start and end of each run. All plasma samples were measured in duplicate and the mean value calculated. All samples for one participant were analysed within the same batch. Inter-assay CVs were 9% and 3% for low and high QCs respectively and intra-assay CVs were ≤ 8% and ≤ 4% respectively.

The ILab uses an enzymatic colorimetric method to measure TAG. First the TAGs are hydrolysed to glycerol and FFA. The glycerol is then phosphorylated to form glycerol-3-phosphate (G3P) which is then oxidised. Hydrogen peroxide is released in this oxidation step. The H$_2$O$_2$ generated reacts with 4-chlorophenol and 4-aminoantipyrine to produce a red quinoneimine dye. The concentration of the dye is determined by measuring the absorbance at 510 nm and is directly proportional to the concentration of TAG in the sample. The chemical reactions are as follows:

\[\text{TAG} \xrightarrow{\text{lipoprotein lipase}} \text{glycerol + FFA}\]
\[\text{glycerol + ATP} \xrightarrow{\text{glycerol kinase}} \text{G3P + ADP}\]
\[\text{G3P + O}_2 \xrightarrow{\text{glycerophosphate oxidase}} \text{dihydroxyacetone phosphate + H}_2\text{O}_2\]
\[\text{H}_2\text{O}_2 + 4\text{-chlorophenol + 4-aminoantipyrine} \xrightarrow{\text{peroxidase}} \text{red quinoneimine + 2H}_2\text{O}\]

2.4.5 Analysis of serum NEFA

Serum NEFA was measured on the ILab 650 analyser (Instrumentation Laboratory, UK), using a Randox Laboratories NEFA ILab 600 kit (Randox, UK). Two QCs were used, Human Control Assay 2 and 3 (Randox, UK) and the calibrant was IL Test ReferrIL G (Instrumentation Laboratory, UK). QCs were measured in triplicate at the start and end of each run. All plasma samples were measured in duplicate and the mean value calculated. All samples for one participant were analysed within the same batch.
batch. Inter-assay CVs were 8% and 6% for low and high QCs respectively and intra-assay CVs were ≤ 7% and ≤ 4% respectively.

The ILab uses an enzymatic colorimetric method to measure NEFA. Firstly NEFA reacts with coenzyme A to form acyl CoA, which is then oxidised and H$_2$O$_2$ released. The H$_2$O$_2$ generated reacts with N-ethyl-N-(2-hydroxy-3-sulphopropyl)-m-toluidine (TOOS) and 4-aminoantipyrine to produce a purple quinoneimine dye. The concentration of the dye is determined by measuring the absorbance at 550 nm and is directly proportional to the concentration of NEFA in the sample. The chemical reactions are as follows:

\[
\text{NEFA} + \text{ATP} + \text{CoA} \xrightarrow{\text{acyl CoA synthase}} \text{Acyl CoA} + \text{AMP} + \text{PPi} \\
\text{Acyl CoA} + \text{O}_2 \xrightarrow{\text{acyl CoA oxidase}} 2,3\text{-trans- enoyl-CoA} + \text{H}_2\text{O}_2 \\
2\text{H}_2\text{O}_2 + \text{TOOS} + 4\text{-aminoantipyrine} \xrightarrow{\text{peroxidase}} \text{purple quinoneimine} + 4\text{H}_2\text{O}
\]

2.4.6 Analysis of plasma insulin

Plasma insulin was measured in two different ways, either by enzyme-linked immunosorbent assay (ELISA) or radioimmunoassay (RIA). The method was however consistent for each study.

**Enzyme-linked immunosorbent assay (ELISA)**

Plasma insulin for the dose-response (Chapter 4) and repeated-dose (Chapter 5) studies was measured by ELISA using a Human Insulin ELISA kit (Millipore, UK). The kit utilises the sandwich ELISA methodology and has no cross-reactivity with intact human pro-insulin or c-peptide.

Unknown samples along with known standards are added to individual wells in a microtitre plate which has been coated with monoclonal mouse anti-human insulin antibodies. The insulin in the samples binds to the antibodies coating the plate. A detection antibody (biotinylated monoclonal mouse anti-human antibody) is added and the plate is incubated for an hour. During this time the detection antibody binds to the captured insulin. The plate is washed to remove any unbound materials and an enzyme solution (horseradish peroxidase) is added, which conjugates with the bound biotinylated detection antibodies. After further incubation the plate is again washed and
substrate solution (3,3',5,5'-tetramethylbenzidine) is added. The substrate reacts with the bound enzyme and a blue colour develops, proportional to the amount of insulin in the sample. The plate is incubated until a strong colour gradient develops in the standard wells. At this point, stop solution (HCl) is added and the samples in the wells change to a yellow colour. The plate is immediately placed in a microplate reader (Opsys MR, Dynex Technologies, US) and the absorbance at 450 nm spectrophotometrically read. The insulin concentration in the unknown samples is derived by plotting the absorbance against the standard curve.

High and low QCs were included at the beginning and end of each run. All samples for one participant were analysed on the same plate. Inter-assay CVs were 3% and 11% for low and high QCs respectively and intra-assay CVs were ≤ 10% and ≤ 6% respectively for the dose-response study (Chapter 3). Inter-assay CVs were 5% and 7% for low and high QCs respectively and intra-assay CVs were ≤ 11% for the repeated-dose study (Chapter 4).

Radioimmunoassay (RIA)

Plasma insulin for the twelve week intervention (Chapters 6 and 7) was measured by radioimmunoassay (RIA) using a Human Insulin Specific RIA kit (Millipore, UK). The kit has very little cross-reactivity with human pro-insulin (< 0.2%). A fixed amount of labelled antigen (\(^{125}\)I-insulin) is incubated with a fixed amount of antibody and the unknown sample. Insulin in the unknown sample competes with the labelled antigen for binding sites; the greater the insulin concentration in the sample, the less labelled antigen that will bind to the antibody. After overnight incubation, precipitating reagent is added and the supernate is aspirated leaving a radioactive pellet in the bottom of the tube. The tubes are then put into a gamma counter and the resultant readings are plotted against a standard curve to determine the insulin concentration of the unknown samples. All samples were measured in duplicate and high and low QCs were included at the beginning, middle and end of each run. All samples for one participant were analysed within the same batch. Inter-assay CVs were 11% for both low and high QCs and intra-assay CVs were ≤ 9% and ≤ 7% for low and high QCs respectively.

2.4.7 Analysis of serum IL-6 and TNF-\(\alpha\)

The inflammatory markers, IL-6 and TNF-\(\alpha\) were analysed by an external laboratory (Clinical Immunology Service, University of Birmingham), using a bead-based multiplex assay (Bio-Plex Precision Pro Human Cytokine Assay kit, Bio-Rad, UK). The plate was analysed on a Luminex-100 plate reader (Bio-Plex Systems, BioRad
Laboratories, California, USA) using a low ‘RP1’ (low PMT - photomultiplier tube) target setting. Data analysis was performed using Bio-Plex Manager 4.1.1 software (Bio-Rad, UK). Intra-assay CVs were < 10%. Limits of detection were reported as 0.46 pg/ml and 0.59 pg/ml for IL-6 and TNF-α respectively. Samples were analysed in duplicate and mean values calculated.

2.5 Salivary caffeine analysis

2.5.1 Saliva collection

Saliva was collected from participants taking part in the 12 week coffee intervention, detailed in Chapter 6. Participants were first asked to thoroughly rinse their mouth with water, to reduce impurities in the sample, before producing more saliva and depositing it into a 20 ml Sterilin Universal container (Fisher Scientific, Loughborough, UK); approximately 1 ml of saliva was collected each time. Samples were frozen at -20 °C for later analysis.

2.5.2 Caffeine analysis

Salivary caffeine analysis was carried out by Dr J. M. Frank at the University of Surrey. The saliva samples collected previously were defrosted and 0.5 ml of saliva was aliquoted into a 2 ml Eppendorf tube, to which 0.5 ml methanol was added. The tubes were vortexed for approximately 30 s and then centrifuged for 10 min at 19071 x g, 4 °C (Eppendorf 5430 R microcentrifuge, Eppendorf UK Ltd) and decanted into sample vials.

Caffeine concentration in the samples was measured in batch by reversed-phase high performance liquid chromatography (HPLC), using a Synergi Hydro ODS (C18) 4.6 x 250 mm column, with 35% methanol in water, run in isocratic mode at 0.75 ml/min; column temperature was 30 °C, injection volume was 20 µl and run time was 10 min. Caffeine content was determined by measuring the absorbance at 260 nm and 280 nm and plotting against a standard curve.

2.6 DNA analysis

DNA was collected from participants in the twelve week intervention (Chapter 6 and 7) to determine whether they were fast or slow caffeine metabolisers as determined by the rs762551 SNP in the CYP1A2 gene.
2.6.1 DNA collection

On the first study day, buccal cells were collected from each participant by wiping a sterile neutral transport swab (Sarstedt, UK) around the inside of both of the participant's cheeks. The swab was left to air-dry for 10 minutes, then frozen at -20 °C for batch analysis at the end of the study.

2.6.2 DNA extraction

MasterAmp DNA extraction solution (Epicentre Biotechnologies, US) was pipetted into 1.5 ml boil-proof microtubes. The tip of each buccal swab was inserted into the extraction solution and swirled and rubbed against the side of the tubes to detach the buccal cells from the swab. The extraction solution acts as a lysing agent to free up the DNA from the cells into the solution. To activate this process, the tubes were vortexed then incubated at 65 °C for 5 min, then vortexed again and incubated at 98 °C for a further 5 min. After a final vortex, the tubes were frozen at -20 °C until they were required.

2.6.3 DNA analysis

The DNA extracted by the method described above was analysed for the rs762551 SNP by a 5'-nuclease allelic discrimination assay (Taqman drug metabolism genotyping assay SNP ID rs762551, gene CYP1A2, Applied Biosystems, UK). The assay is performed in three stages:

1. A reaction mixture is prepared and pipetted onto a microplate along with the DNA sample
2. The DNA is amplified by a polymerase chain reaction (PCR)
3. The plate is read and SNPs identified by a specialist software program

Reaction Mix

The reaction mix was prepared by adding master mix (Taqman Genotyping Master Mix, Fisher Scientific, UK), assay (Taqman rs762551 as described previously) and nuclease-free water to a 1.5 ml tube. The components were added in the following ratios:

Master mix: 6.25 µL/sample
Assay: 0.625 µL/sample
H₂O: 4.625 µL/sample
The reaction mix was mixed thoroughly then 11.5 µL was pipetted into each well of a microplate along with 1 µL of DNA sample. The plate was covered, vortexed and centrifuged for 10 min at 1109 x g (Allegra 21R centrifuge, Beckman Coulter, US).

**PCR stage**

The plate containing the reaction mix and samples was then inserted into an Applied Biosystems 7500 Real Time PCR system and the PCR was run. The PCR was set up with the following stages:

- Pre-PCR read (holding stage): 1 min at 60 °C
- Holding stage/enzyme activation: 10 min at 95 °C
- Cycling stage (40 cycles):
  - Denaturation: 15 sec at 95 °C
  - Annealing/extension: 1 min at 60 °C
- Post-PCR read (holding stage): 1 min at 60 °C

The Taqman assay contains forward and reverse PCR primers for amplifying the sequence of interest on the DNA and two labelled minor groove binder (MGB) probes. Each probe has a fluorescent reporter dye at the 5’ end of the probe (VIC dye on the allele 1 probe; FAM dye on the allele 2 probe) and a nonfluorescent quencher (NFQ) at the 3’ end. The SNP of interest is situated in the middle of each probe. The reporter dye is quenched if the probe is intact.

PCR consists of many cycles of denaturation at high temperatures and annealing/extension at lower temperatures in order to amplify the DNA regions of interest. During the denaturation phase the double stranded DNA is separated into single strands. In the annealing phase the probes anneal only to complementary sequences on the single stranded DNA; DNA polymerase then cleaves the reporter dye from the probe resulting in an increase in fluorescence of that dye. Thus, an increase in fluorescence of either VIC or FAM dye indicates homozygosity for the corresponding allele. An increase in fluorescence of both indicates heterozygosity.

**Plate reading**

The fluorescence of each individual dye is read at the beginning and end of the PCR phase and the difference in readings is calculated by the PCR software (Applied Biosystems 7500 Real Time PCR system v 2.06). The software then uses these values to plot an allelic discrimination plot with ΔVIC on the x-axis and ΔFAM on the y-axis, allowing identification of the alleles present in each sample.
2.7 Estimation of insulin sensitivity and beta cell function

The Homeostasis Model of Assessment (HOMA) was used to assess fasting insulin sensitivity (HOMA2_%S) and fasting beta cell function (HOMA2_%B), using the HOMA2 calculator, version 2.2.3 (University of Oxford, UK).

The Matsuda Index (Matsuda & DeFronzo, 1999) was calculated as a measure of postprandial insulin sensitivity, according to the following equation:

\[
\sqrt{\frac{10,000}{(\text{fasted glucose} \times \text{fasted insulin}) \times \text{mean glucose} \times \text{mean insulin during OGTT})}
\]

The insulin:glucose AUC ratio (AUC\(_I/G\)) was calculated as a measure of postprandial insulin secretion and the product of AUC\(_I/G\) and Matsuda Index was used as an indirect marker of postprandial beta cell function (169).

2.8 Statistical analysis

All statistical analysis was carried out using SPSS Statistics software, version 22 (Chicago, USA). The Shapiro-Wilks test was used to check normality of data (170). Repeated measures analysis of variance (RM ANOVA) and paired and unpaired t tests were used for comparisons between and within groups where data were normally distributed. A Bonferroni adjustment for multiple comparisons was applied when post hoc analysis was used. Non-normally distributed data were log transformed and re-tested for normality. If a dataset contained values less than one then a fixed value was added to all data in the set (calculated to make the smallest value equal to one) prior to log transformation (171). If found to be normally distributed after log transformation, parametric tests were carried out on the log transformed data. Equivalent non-parametric tests were used where the data were not normally distributed. Fisher’s Exact tests were used for assessment of independence of variables. The incremental area under curve (IAUC) and total area under curve (AUC) for glucose and insulin for each treatment were calculated by the trapezoid method with area under baseline ignored for calculation of IAUC as recommended by the Food and Agriculture Organization (172) and described by Brouns et al. (173). Statistical significance was taken as \( p < 0.05 \).
Chapter 3. Systematic review on the acute effects of coffee on postprandial glucose response

3.1 Introduction

Epidemiological evidence suggests an inverse association between the amount of coffee consumed and the development of T2DM (2,87,174). This appears to hold true for both CC and DC (6). Intervention studies into the acute effects of coffee on glycaemic control are, however, not so conclusive and are often contradictory.

Whilst systematic reviews have been conducted on the epidemiological data (175) and on the acute effects of caffeine (176), there has been no systematic review of the acute effects of coffee to date. This is of particular importance when it is considered that poor glycaemic control is a key risk factor for development of complications in T2DM (177). There are also currently no guidelines in the UK regarding coffee consumption for those with T2DM, perhaps because the evidence is unclear.

3.2 Aims

To perform a systematic review of all randomized controlled trials (RCTs) that compared the effects of coffee to a control drink on either an OGTT or CHO-containing meal in human populations. CC and DC were examined separately.

3.3 Methods

The Web of Science (all databases, including Medline) was searched on 9th October 2015 with the following search criteria:

- TOPIC: (human AND (coffee OR caffeine) AND (glucose OR glycemic OR glycaemic OR glycemia OR glycaemia))
- Refined by: [excluding] DOCUMENT TYPES: (BOOK OR PATENT OR CASE REPORT OR EDITORIAL OR LETTER)
- Timespan: All years.
- Search language=Auto

To minimise publication bias, the following online resources were also searched on 28th October 2015: ClinicalTrials.gov (http://www.clinicaltrials.gov), ICTRP: International Clinical Trials Registry Platform Search Portal WHO (http://apps.who.int/trialsearch/Default.aspx) and Open SIGLE: System for Information
on Grey Literature in Europe (http://opensigle.inist.fr). The search terms ‘CAFFEINE’ or ‘COFFEE’ and ‘GLUCOSE’ were used.

The searches produced 1048 results, including 22 duplicates. Based on the titles and abstracts 1018 were excluded, leaving 30 for more detailed examination. Papers excluded were either not relevant, measured caffeine instead of coffee, had no control or were not acute interventions; several were longer-term interventions and many were review papers or cohort studies. One relevant unpublished trial was found on clinicaltrials.gov; when the authors were contacted they reported that the trial had never started and had been withdrawn. The references for each of the 30 shortlisted papers along with the review papers were examined for further potential studies, but no further studies were identified.

Of the 30 selected for detailed examination, three early studies were excluded as they were foreign language papers and no English translations were available. A further seven were rejected for reasons reported in Figure 3.3-1. This left 20 studies to be included in the review.
3.4 Results

The 20 studies selected for inclusion in the review are summarised in Table 3.4-1.
### Table 3.4-1  Studies selected for inclusion in systematic review.

<table>
<thead>
<tr>
<th>Study</th>
<th>n</th>
<th>Subject characteristics</th>
<th>Regular coffee intake</th>
<th>Washout period</th>
<th>OGTT/meal</th>
<th>Meal concurrent/ delayed</th>
<th>Capillary/ venous samples</th>
<th>Dose/ preparation</th>
<th>Caffeine per kg BW (mg/kg)</th>
<th>Absolute amount of caffeine (mg)</th>
<th>Effect on blood glucose</th>
<th>Effect on blood insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feinberg et al., 1968</td>
<td>23</td>
<td>15 M, 8 F</td>
<td>Healthy</td>
<td>N/A</td>
<td>None specified</td>
<td>1 g glucose/kg BW in 400 ml water + lemon juice</td>
<td>Concurrent</td>
<td>Unspecified</td>
<td>CC: 5 g instant PL: water (total volume 400 ml per treatment)</td>
<td>N/A</td>
<td>220</td>
<td>CC&gt;PL at 30 min/ 60 min</td>
</tr>
<tr>
<td>Young and Wolver, 1998</td>
<td>12</td>
<td>6 M, 6 F</td>
<td>Healthy, 26 ± 1.9 y BMI 22.8 ± 1.0 kg/m²</td>
<td>N/A</td>
<td>None specified</td>
<td>Mixed meal containing 50 g CHO, 11.7 g fat, 11.8 g protein</td>
<td>Concurrent</td>
<td>Capillary</td>
<td>CC: 1 rounded tsp instant coffee PL: water (total volume 250 ml per treatment)</td>
<td>N/A</td>
<td>N/A</td>
<td>CC&gt;PL at 45 min/ 60 min no effect on IAUC</td>
</tr>
<tr>
<td>Johnston et al., 2003</td>
<td>9</td>
<td>4 M, 5 F</td>
<td>Healthy, 26 ± 3.2 y BMI ≤ 25 kg/m²</td>
<td>N/A</td>
<td>No caffeine the evening before each study day</td>
<td>25 g glucose in 400 ml water</td>
<td>Concurrent</td>
<td>Venous</td>
<td>CC: 8.7 g instant DC: 12 g instant (both providing 353 mg CQA) PL: water (total volume 400 ml per treatment)</td>
<td>N/A</td>
<td>N/A</td>
<td>CC: no effect over 180 min CC &gt; DC and PL for 30 min AUC, DC: no effect</td>
</tr>
<tr>
<td>Battram et al., 2006</td>
<td>11 M</td>
<td>Healthy</td>
<td>76.4 ± 1.9 kg</td>
<td>10/11 non-caffeine users Caffeine-free for 7 days prior to study and throughout</td>
<td>75 g glucose OGTT + 60 min</td>
<td>Venous</td>
<td>CC: drip-filtered, unspecified volume giving 4.45 mg/kg caffeine DC: equal volume to CC PL: 4.45 mg/kg dextrose</td>
<td>4.45</td>
<td>Mean 340</td>
<td>Control &gt; DC IAUC, trend for CC &gt; DC PL&gt;DC at 60/90 min</td>
<td>Trend for CC &gt; DC (IAUC)</td>
<td></td>
</tr>
<tr>
<td>Thom, 2007</td>
<td>12</td>
<td>Healthy BMI &lt; 25 kg/m²</td>
<td>N/A</td>
<td>None specified</td>
<td>25 g glucose in 400 ml water</td>
<td>Concurrent</td>
<td>Unspecified</td>
<td>CC: 10 g instant DC: 10 g instant PL: water (total volume 400 ml per treatment)</td>
<td>N/A</td>
<td>N/A</td>
<td>CC&gt;DC: no effect on AUC</td>
<td>Not measured</td>
</tr>
</tbody>
</table>

AUC: area under curve; BMI: body mass index; BW: bodyweight; CC: caffeinated coffee; CHO: carbohydrate; CF: caffeine; CQA: caffeoylquinic acids; DC: decaffeinated coffee; DR: dark roast; F: female; GI: glycaemic index; IAUC: incremental area under curve; LR: light roast; Lrg: large; M: male; Med: medium; N/A: not available; OGTT: oral glucose tolerance test; PL: placebo/control; RM ANOVA: repeated measures analysis of variance; SML: small; T2DM: type 2 diabetes mellitus
### Table 3.4-1 cont. Studies selected for inclusion in systematic review.

<table>
<thead>
<tr>
<th>Study</th>
<th>n</th>
<th>Subject characteristics</th>
<th>Regular coffee intake</th>
<th>Washout period</th>
<th>OGGT/meal</th>
<th>Meal concurrent/delayed</th>
<th>Capillary/venous samples</th>
<th>Dose/preparation</th>
<th>Caffeine per kg BW (mg/kg)</th>
<th>Absolute amount of caffeine (mg)</th>
<th>Effect on blood glucose</th>
<th>Effect on blood insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Louie et al., 2008</td>
<td>8</td>
<td>Healthy, 20 - 35 y BMI 17.6 - 24.3 kg/m²</td>
<td>≥ 1 cup/day</td>
<td>None specified</td>
<td>586 g instant mashed potato (75 g available CHO)</td>
<td>+ 60 min</td>
<td>Capillary</td>
<td>CC: 4 g instant; DC: 4 g instant; PL: water (total volume 250 ml per treatment)</td>
<td>N/A</td>
<td>150</td>
<td>CC &gt; PL: glucose score; CC &gt; DC: glucose score</td>
<td></td>
</tr>
<tr>
<td>Aldughassi and Wolever, 2008</td>
<td>10</td>
<td>Healthy, 31 ± 10 y BMI 25 ± 4.7 kg/m²</td>
<td>N/A</td>
<td>None specified</td>
<td>One of 3 meals (each containing 50 g available CHO): 109 g white bread; 84 g cheese puffs; 64 g fruit leather each with or without tea/coffee</td>
<td>Concurrent</td>
<td>Capillary</td>
<td>DC: 250 ml drip-filtered ground (1 tbsp ground coffee to 300 ml water); Tea: 1 teabag steeped for 30 s in 250 ml boiling water; PL: 250 ml water</td>
<td>N/A</td>
<td>N/A</td>
<td>CC + tea &gt; PL at 30 min for WB and CP, but not FL no diff in IAUC/GI</td>
<td></td>
</tr>
<tr>
<td>van Dijk et al., 2009</td>
<td>15</td>
<td>Healthy, 39.9 ± 16.5 y BMI 25 - 35 kg/m²</td>
<td>Coffee drinkers max 1 cup/day from 1 week prior then no coffee on the day before each visit</td>
<td>75 g glucose OGTT</td>
<td>+30 min</td>
<td>Venous</td>
<td>DC: 12 g instant (providing 264 mg CGA); PL: 1 g mannitol (both dissolved in 270 ml water)</td>
<td>N/A</td>
<td>N/A</td>
<td>DC: no effect on individual time-points or AUC; DC: no effect on individual time-points or AUC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Greenberg et al., 2010</td>
<td>11</td>
<td>Healthy, 23.5 ± 5.7 y BMI 23.6 ± 4.2 kg/m²</td>
<td>N/A</td>
<td>No caffeine from 1 week prior to 1st visit</td>
<td>75 g glucose OGTT</td>
<td>+60 min</td>
<td>Venous</td>
<td>CC: 500-600 ml drip-filtered ground (40 g ground CC to 8 cups water); DC: same volume as CC (57 g ground DC to 8 cups water); PL: water</td>
<td>6</td>
<td>N/A</td>
<td>PL &lt; DC/CC at 10 min/30 min</td>
<td></td>
</tr>
<tr>
<td>Moisey et al., 2010</td>
<td>10</td>
<td>Healthy, 20-27 y BMI 23.9 ± 0.8 kg/m²</td>
<td>3/10 non coffee drinkers, others (1-3 cups/day) No caffeine for 48 h before each session</td>
<td>Cereal and skimmed milk (75 g available CHO)</td>
<td>Concurrent</td>
<td>Venous</td>
<td>CC: 535-812 ml drip filtered ground DC: same volume as CC; PL: water</td>
<td>5</td>
<td>Mean 398</td>
<td>PL &lt; DC/CC iAUC 3 hr PL &lt; DC &lt; CC at 180 min No effect on IAUC</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

AUC: area under curve; BW: bodyweight; CC: caffeinated coffee; CHO: carbohydrate; CF: caffeine; CQA: caffeoylquinic acids; DC: decaffeinated coffee; DR: dark roast; F: female; GI: glycaemic index; IAUC: incremental area under curve; LR: light roast; Lrg: large; M: male; Med: medium; N/A: not available; OGGT: oral glucose tolerance test; PL: placebo/control; RM ANOVA: repeated measures analysis of variance; SML: small; T2DM: type 2 diabetes mellitus
Table 3.4 cont. Studies selected for inclusion in systematic review.

<table>
<thead>
<tr>
<th>Study</th>
<th>n</th>
<th>Subject characteristics</th>
<th>Regular coffee intake</th>
<th>Washout period</th>
<th>OGTT/meal</th>
<th>Meal concurrent/ delayed</th>
<th>Capillary/ venous samples</th>
<th>Dose/ preparation</th>
<th>Caffeine per kg BW (mg/kg)</th>
<th>Absolute amount of caffeine (mg)</th>
<th>Effect on blood glucose</th>
<th>Effect on blood insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gavrieli et al., 2011</td>
<td>16 M</td>
<td>Healthy 21-39 y BMI 25.5 ± 2.3 kg/m²</td>
<td>1.3 ± 1.0 servings/day</td>
<td>No caffeine for 3 days before each session</td>
<td>1 slice white bread, 5 g butter, 10 g sugar (~ 24 g CHO)</td>
<td>Concurrent</td>
<td>Venous</td>
<td>CC: instant DC: instant same amount of coffee as CC</td>
<td>3</td>
<td>200-280</td>
<td>CC&gt;PL at 60 min no effect on AUC or IAUC DC: no effect</td>
<td></td>
</tr>
<tr>
<td>Beaudoin et al., 2011</td>
<td>10 M</td>
<td>Healthy 22.9 ± 0.4 y 78.9 ± 4.8 kg BMI 24.7 ± 0.8 kg/m²</td>
<td>N/A</td>
<td>No caffeine for 48 h before each session</td>
<td>75 g dextrose OGTT (6 h after 1 g lipid/kg BW)</td>
<td>+ 60 min</td>
<td>Venous</td>
<td>CC/DC: drip-filtered, unspecified volume PL: water</td>
<td>5</td>
<td>Mean 395</td>
<td>CC&gt;DC/PL at 60 min no effect on IAUC CC&gt;PL at 60 min CC&gt;DC at 90 min No effect on IAUC</td>
<td></td>
</tr>
<tr>
<td>Hätonen et al., 2012</td>
<td>12</td>
<td>Healthy 34.8 ± 10.4 y BMI 21.9±2.5 kg/m²</td>
<td>Habitual coffee drinkers, mean intake 392 ml/day filter coffee</td>
<td>None specified</td>
<td>50 g glucose</td>
<td>Concurrent</td>
<td>Capillary</td>
<td>Sml CC: filter coffee made with 7 g grounds Lrg CC: filter coffee made with 14 g grounds PL: water (all made up to total volume 550 ml)</td>
<td>N/A</td>
<td>Sml: 151 Lrg: 303</td>
<td>No effect on IAUC did not compare timepoint data No effect on IAUC did not compare timepoint data</td>
<td></td>
</tr>
<tr>
<td>Krebs et al., 2012</td>
<td>18</td>
<td>T2DM 43 - 82 y</td>
<td>At least 3 cups/week (30-540 mg CF/day)</td>
<td>No caffeine for 24 h before each session</td>
<td>75 g glucose OGTT</td>
<td>+ 60 min</td>
<td>Venous</td>
<td>CC: double espresso DC: double espresso PL: water (same unspecified volume)</td>
<td>N/A</td>
<td>180 (approx)</td>
<td>CC &gt; DC/PL AUC (trend) Did not compare timepoint data</td>
<td></td>
</tr>
</tbody>
</table>

AUC: area under curve; BMI: body mass index; BW: bodyweight; CC: caffeinated coffee; CHO: carbohydrate; CF: caffeine; CQA: caffeoylquinic acids; DC: decaffeinated coffee; DR: dark roast; F: female; GI: glycaemic index; IAUC: incremental area under curve; LR: light roast; Lrg: large; M: male; Med: medium; N/A: not available; OGTT: oral glucose tolerance test; PL: placebo/control; RM ANOVA: repeated measures analysis of variance; SML: small; T2DM: type 2 diabetes mellitus
Table 3.4-1 cont. Studies selected for inclusion in systematic review.

<table>
<thead>
<tr>
<th>Study</th>
<th>n</th>
<th>Subject characteristics</th>
<th>Regular coffee intake</th>
<th>Washout period</th>
<th>OGTT/meal</th>
<th>Meal concurrent/ delayed</th>
<th>Capillary/ venous samples</th>
<th>Dose/ preparation</th>
<th>Caffeine per kg BW (mg/kg)</th>
<th>Absolute amount of caffeine (mg)</th>
<th>Effect on blood glucose</th>
<th>Effect on blood insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaabi et al., 2013</td>
<td>23</td>
<td>13 healthy</td>
<td>N/A</td>
<td>None specified</td>
<td>Gi study: Khala dates (50 g available CHO) with or without coffee versus 50 g glucose</td>
<td>Coffee served immediately after dates</td>
<td>Capillary</td>
<td>CC: 100 ml (from 5 tbsp medium roast ground Arabic coffee boiled with 1000 ml water then filtered) PL: water (all made up to total volume 250 ml)</td>
<td>N/A</td>
<td>N/A</td>
<td>No difference in GI</td>
<td>Not measured</td>
</tr>
<tr>
<td>Gavrieli et al., 2013</td>
<td>33</td>
<td>≥ 1 cup/day</td>
<td>No caffeine for 24 h before each session</td>
<td>1 slice white bread, 5 g butter, 10 g sugar (~ 24 g CHO)</td>
<td>Concurrent Venous</td>
<td>Sml CC: mean 6.0 g instant Lrg CC: mean 11.9 g instant</td>
<td>PL: water</td>
<td>Sml: 3 Lrg: 6</td>
<td>Mean 228 Mean 457</td>
<td>Sml/Lrg &gt; PL for IAUC interaction*time effect Lrg &gt; PL at 60/90/120 min Lrg &lt; PL at 15/30 min Lrg &lt; PL at 60 min Sml &lt; PL at 15 min Lrg &lt; Sml at 15 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Al-Mssallem and Brown, 2013</td>
<td>10</td>
<td>Healthy</td>
<td>N/A</td>
<td>None specified</td>
<td>Gi study: Khulas dates (50 g available CHO) with or without coffee versus 50 g glucose</td>
<td>Concurrent Capillary</td>
<td>CC: Arabic coffee: amount/volume unspecified PL: water</td>
<td>N/A</td>
<td>N/A</td>
<td>Trend for difference in IAUC overall (p=0.08). No post hoc tests</td>
<td>No difference in IAUC</td>
<td></td>
</tr>
<tr>
<td>Schubert et al., 2014</td>
<td>12</td>
<td>Healthy</td>
<td>22-742 mg/day caffeine</td>
<td>No coffee for 36 h before each session</td>
<td>Concurrent Venous</td>
<td>One dose with meal, second dose + 120 min</td>
<td>DC: 5 g instant CC: 5 g instant DC plus 2mg/kg CF capsule PL: psyllium capsule 250 mg (all with 225 ml water) N.B. Treatment given twice: at T0 then repeated at T120</td>
<td>4</td>
<td>Mean 278</td>
<td>No effect on AUC Did not compare timepoint data</td>
<td>Not measured</td>
<td></td>
</tr>
</tbody>
</table>

AUC: area under curve; BMI: body mass index; BW: bodyweight; CC: caffeinated coffee; CHO: carbohydrate; CF: caffeine; CQA: caffeoylquinic acids; DC: decaffeinated coffee; DR: dark roast; F: female; GI: glycaemic index; IAUC: incremental area under curve; LR: light roast; Lrg: large; M: male; Med: medium; N/A: not available; OGTT: oral glucose tolerance test; PL: placebo/control; RM ANOVA: repeated measures analysis of variance; SML: small; T2DM: type 2 diabetes mellitus
### Table 3.4-1 cont.  Studies selected for inclusion in systematic review.

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<tr>
<th>Study</th>
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<th>Subject characteristics</th>
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<th>Washout period</th>
<th>OGTT/meal</th>
<th>Capillary/venous samples</th>
<th>Dose/ preparation</th>
<th>Caffeine per kg BW (mg/kg)</th>
<th>Absolute amount of caffeine (mg)</th>
<th>Effect on blood glucose</th>
<th>Effect on blood insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Robertson et al., 2015</td>
<td>10 M</td>
<td>Healthy Overweight</td>
<td>2.3 ± 1.6 servings/day</td>
<td>No caffeine for 48 h before each session</td>
<td>50 g glucose</td>
<td>Concurrent</td>
<td>Capillary</td>
<td>Sml CC: 2 g instant (47 mg CQA)</td>
<td>Sml: 100 Med: 200 Lrg: 400</td>
<td>Sml &gt; PL for IAUC</td>
<td>No effect overall, RM ANOVA or IAUC</td>
</tr>
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<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rakvaag and Dragsted, 2015</td>
<td>11</td>
<td>Healthy</td>
<td>N/A</td>
<td>No caffeine for 48 h before each session</td>
<td>75 g glucose in 300 ml water with citric acid</td>
<td>+ 30 min</td>
<td>Venous</td>
<td>LR CC: 300 ml (taken from 45 g light roast ground coffee made in cafetiere with 750 ml water), providing 399.6 mg CQA, DR CC: 300 ml (as for LR, but using dark roasted beans), providing 66.6 mg CQA</td>
<td>Mean: 2</td>
<td>DR &gt; PL, RM ANOVA and Interaction*time effect</td>
<td>DR/LR &gt; PL at 60 min</td>
</tr>
</tbody>
</table>

**AUC:** area under curve; **BMI:** body mass index; **BW:** bodyweight; **CC:** caffeinated coffee; **CHO:** carbohydrate; **CF:** caffeine; **CQA:** caffeoylquinic acids; **DC:** decaffeinated coffee; **DR:** dark roast; **F:** female; **GI:** glycaemic index; **IAUC:** incremental area under curve; **LR:** light roast; **Lrg:** large; **M:** male; **Med:** medium; **N/A:** not available; **OGTT:** oral glucose tolerance test; **PL:** placebo/control; **RM ANOVA:** repeated measures analysis of variance; **SML:** small; **T2DM:** type 2 diabetes mellitus
3.4.1 Participant characteristics

The number of participants in each study ranged from 8 to 33. Most studies (18/20) were carried out on healthy participants, with only two studies examining the effects of coffee on individuals with T2DM \(^{(110,181)}\). Mean age of participants was generally under 35 y with the exception of the T2DM studies, where the participants were older (mean 40.8 y and 43 - 82 y). Half of the studies did not specify whether their participants were habitual coffee drinkers or not; of those that did provide this information, 8/10 used habitual coffee drinkers.

3.4.2 Methodology

Of the twelve studies that reported one, the pre-study washout period ranged from no caffeine the evening before the study \(^{(147)}\) to a requirement to be caffeine-free for 7 days prior and to remain caffeine-free for the duration of the study \(^{(10)}\). Half gave glucose, ranging from 25 to 75 g, dissolved in water (200 - 400 ml) as their CHO load, with the remainder giving mixed meals or individual foodstuffs providing between 24 and 75 g of available CHO. Where a mixed meal was given, the accompanying coffee ranged in volume from 200 - 812 ml. One study did not specify the content of their test meal \(^{(182)}\). Coffee was given concurrently with the glucose/meal in 65% of cases and up to 1 h beforehand in the remainder.

Seven took capillary blood samples via the finger-prick technique, eleven took venous samples and two did not specify the collection method. Blood samples were taken over various amounts of time ranging from 2 h to 4.5 h. Frequency was every 15 - 30 min in most cases, with one study measuring hourly after the first 15 min \(^{(182)}\).

Twelve studies did not perform a simple comparison of CC/DC and control, but instead included other treatments. Of these, three also tested caffeine \(^{(10,126,182)}\), four tested different strengths or roasts of coffee \(^{(183–186)}\) and four tested other miscellaneous items such as CGAs, tea, different volumes of water, iced buns, sugar and cola \(^{(9,144,146,187)}\). One employed a lipid preload 5 h before the OGTT and had two other comparisons not included here: OGTT without preload and preload without OGTT \(^{(7)}\). One study gave CC to five participants and tea to another five and combined the results stating that both treatments gave similar results \(^{(188)}\).

3.4.3 Coffee dose

Type of coffee varied between studies, with ten giving their participants instant coffee, six giving drip-filtered coffee, two using Arabic coffee, one using a French press and
one giving espresso coffee. Most gave a single dose of coffee after an overnight fast, however one gave a lipid preload 5 h before the OGTT \(^7\) and another gave their coffee dose in two boluses, one at 0 min and the other at 120 min \(^{182}\). Seven chose their coffee dose to deliver a set amount of caffeine per kg BW. Amounts given varied from 3 - 6 mg/kg. Those giving instant coffee, gave doses in the range of approximately 2 g up to 12 g. Seven studies did not report the amount of caffeine in their dose; of those that did report it, the lowest dose was 100 mg and the highest stated a mean dose of 526 mg. Only one study examined DC versus a control, without also looking at the effects of CC \(^{144}\). Of the remainder, ten compared CC, DC and a control and the rest did not measure the effects of DC.

### 3.4.4 Caffeinated coffee

**Effect on blood glucose**

Thirteen studies found CC increased postprandial glucose more than a control drink, although only five of these found a significant effect on the entire postprandial period; the remaining eight found either CC produced higher glucose than control at one or more individual time-points or reported a trend for an overall effect.

Moisey *et al.* reported that both CC and DC increased the 3 h postprandial glucose IAUC more than control \(^8\); all three treatments showed very similar results until 90 min post treatment, at which point both DC and control had returned to baseline. Between 90 and 180 min, both DC and control dropped below baseline and remained there, whereas CC glucose dropped more slowly reaching baseline by 150 min. Gavrieli *et al.* demonstrated an 11.4% greater increase for their higher dose (6 mg/kg) and 8.1% for their lower dose (3 mg/kg), with the main difference occurring between 60 and 120 min \(^{184}\). Similarly to Moisey *et al.*, their control treatment dipped below baseline in this time-frame.

In contrast, Louie *et al.* demonstrated a 28% increase in the glucose score (calculated as the ratio of IAUC after treatment to the control IAUC) following CC ingestion, with the increase being most apparent between 30 and 90 min \(^9\). They also appeared to show an increase in the glucose peak, although this was not tested statistically and appears to be a very small increase (in the region of +0.5 mmol/L). Similarly, Robertson *et al.* observed the main differences to be between 30 and 90 min, with no dip below baseline and a difference of 17% (1.4 mmol/L) in peak values \(^{185}\). They also reported a time * treatment interaction, with coffee delaying the time to peak versus control. Rakvaag and Dragsted found the main differences to be between 30 and 120 min, with
no dip below baseline, and reported CC to produce higher glucose than control, post-peak, at 60 min\(^{(186)}\), indicating a similar response pattern to both Louie \textit{et al.} and Robertson \textit{et al.}

In contrast to all others, one early study\(^{(108)}\) observed less of an increase in glucose at 30 and 60 min following CC consumption compared to their control, with values being 15% and 19%, respectively, lower. They did not compare overall response. It was the only study to vary the CHO load according to bodyweight, although it kept the coffee dose static (5 g instant CC) for all participants.

When the studies comparing time-point data are examined in more detail, 3/11 found that CC increased the glucose peak \textit{versus} control; these three increased the peak value by between 1 mmol/L\(^{(147)}\) and 2 mmol/L\(^{(7)}\). Of the remainder, two raised pre-peak values, five increased post-peak values and one reduced them.

\textit{Effect on blood insulin}

Of those that measured insulin, 12/14 found no overall effect of CC \textit{versus} control; One found CC increased insulin \textit{versus} control for AUC and various individual time-points\(^{(126)}\), and another found CC increased insulin overall, but not at any individual time-points\(^{(186)}\). Gavrieli \textit{et al.} found an interaction \* time effect with CC reducing insulin \textit{versus} control at 15 min but increasing it at 60 min, resulting in the insulin response curve shifting to the right\(^{(184)}\). Two further studies reported increased insulin following CC at a single time-point only\(^{(7,8)}\).

3.4.5 Decaffeinated coffee

\textit{Effect on blood glucose}

Nine studies found no overall effect of DC \textit{versus} a control; one found DC worsened glucose response over the entire time period\(^{(8)}\) and one found an improvement\(^{(10)}\). When individual time-points were examined, three studies found no effect of DC\(^{(7,144,161)}\), one found greater glucose compared to placebo at two time-points\(^{(126)}\) and one found less of an increase in glucose for DC at two time-points\(^{(10)}\).

Louie \textit{et al.} found CC produced a larger glucose response than DC overall\(^{(9)}\) and two others found a trend for the same\(^{(10,110)}\); three found CC to produce higher blood glucose than DC at specific time-points\(^{(7,8,147)}\).

\textit{Effect on blood insulin}

Nine studies measured the effects of DC on insulin; seven of these found no effect. Of the remaining two, one found DC produced a larger AUC than control\(^{(126)}\) and the other
found no overall effect but reported DC to raise insulin more than control immediately after meal ingestion \(^{8}\).

Four of eight studies, that measured both CC and DC, reported CC to produce a larger insulin response than DC, either over the whole time-course or at individual time-points \(^{7,9,10,147}\).

### 3.5 Discussion

#### 3.5.1 Effects of caffeinated coffee

Of the five studies that found an overall greater increase in glucose response following CC ingestion, in two cases the difference was mainly due to the control drink dipping below baseline. It is debatable whether CC could be said to have a detrimental effect on glucose in these instances, although it could be argued that because CC extended the time taken for glucose to return to fasting levels, then that in itself is a detrimental effect.

It has been suggested that the increase observed in postprandial glycaemic response following CC consumption can be attributed primarily to caffeine, as caffeine in isolation has been demonstrated to increase the postprandial blood glucose response more than CC \(^{10}\). Caffeine reaches peak levels in the blood approximately 30 min to one hour after ingestion and then slowly disappears with a half-life of about 5 h \(^{79}\). It could be hypothesised, therefore, that studies giving CC in advance of a meal, would result in a greater increase in the postprandial glucose response in comparison with studies giving CC concurrently with a meal, as a result of higher blood caffeine concentrations. Several studies in this review chose to give their CC up to 60 min before the meal to maximise the effects of caffeine, however this does not appear to have resulted in an increased glycaemic excursion in their participants in comparison with the other studies. Furthermore, four out of the five studies which reported an effect of CC over the entire postprandial period gave their dose concurrently with the meal.

It is interesting that Feinberg et al. found CC reduced the postprandial increase in glucose compared to control, however, given that all other studies showed either a worsening or no effect of CC on postprandial glucose, it is likely that this result is due to chance rather than any genuine beneficial effect.

Although the majority of studies (13/19) found some sort of worsening of the glucose response versus a control, in many cases the effect was only found at individual time-points, which may not be statistically valid or clinically relevant. This is in contrast
to a recent systematic review on the acute effects of caffeine in T2DM which found caffeine worsened postprandial glucose in all cases, both overall and at multiple time-points \(^{(176)}\). This difference may be due to caffeine producing a greater effect than coffee, but equally may be due to the populations studied as those with IGT, such as is found in T2DM, may be more susceptible to the effects of CC.

### 3.5.2 Effects of decaffeinated coffee

Despite giving very high doses of DC in some cases (up to 12 g instant), over 80\% of the studies found no overall difference in the glucose response compared to a control, with the remaining two, both of which gave relatively high doses, being split between showing an improvement and a worsening. A similar result was found for insulin with 7/9 showing no overall difference between DC and control.

When CC was compared with DC, the rise in glucose and insulin was generally found to be lower for DC than CC, both overall and at specific time-points, but it is not clear whether other research teams performed the comparison and simply did not report it due to inherent publication bias.

It would appear from these results that DC has no effect on postprandial glucose, either positive or negative. This is perhaps surprising given that it has been suggested that bioactive components in coffee, such as CGAs, may have a beneficial effect and may attenuate the acute negative effects of caffeine on glycaemia. Studies involving CGA on its own \(^{(144)}\) and coffee enriched with CGA \(^{(146)}\) have both demonstrated a reduction in glucose response versus control. The lack of effect of DC in the studies examined here might be explained by insufficient amounts of CGA in the coffees tested. The CGA content of coffee can vary considerably \(^{(76)}\), however the majority of these studies did not measure CGA content. Of the two that did, both gave DC containing less total CGA than that usually found to produce an effect; Johnston \textit{et al.} gave 353 mg and van Dijk \textit{et al.} gave 264 mg total CQA (the main CGA in coffee), compared to approximately 700 mg given by Thom and 1000 mg by van Dijk \textit{et al.} However, Jokura \textit{et al.} reported a reduction in postprandial glucose peak following GCE at a lower level of 355 mg total CQA, but with a higher number of participants (n=19) \(^{(145)}\).

### 3.5.3 Methodology

Whilst most of the populations studied were relatively young, healthy people, the study designs themselves were quite heterogeneous. Glucose doses varied considerably (25 - 75 g), as did the CHO content of mixed meals. Several different types of coffee
were used with a wide range of caffeine doses and the dosing regimen varied from concurrent dosing to 60 min in advance of the CHO load. It is perhaps not surprising then that the results of the studies also varied.

However, there does not appear to be any link between the results obtained and any of these parameters. Of the seven studies that found an overall increase (or trend for an increase) in glucose response with CC, there appears to be no consistency in the study design. The amount of available CHO ranged from 24 to 75 g and was from both glucose and mixed meals. The amount of caffeine ranged from 100 to 457 mg, including the lowest and highest doses of all the included studies, and number of participants ranged from 8 to 33.

It is possible that the lower doses of CHO (24 / 25 g) were insufficient to produce an overall response in most cases here, as 3 of 4 studies employing this lower amount did not see an overall effect and the one which did had greater numbers (n=33) increasing its power to detect a smaller difference between treatments. A typical 2000 kcal/day food intake should consist of 250 g CHO according to current guidelines; this equates to a mean intake of 83 g CHO per meal over the course of a day. Therefore, studies utilising a CHO dose of 25 g are not particularly representative of real-life behaviour.

It has been suggested that 10 participants are sufficient for glycaemic index (GI) testing, however, as an individual’s response to a glucose load is known to vary from day to day, it is recommended that the reference food (glucose or white bread) is repeated twice. Six of the selected studies tested their reference food more than once; one also measured their test drinks more than once. Two of the five that found an overall effect on glucose measured their control more than once. One of these had the lowest number of participants and lowest CC dose, so it is likely that this will have increased the power of that particular study. Four other studies measured their control more than once and did not observe an overall effect; two of these reported differences at some time-points, the others did not compare individual time-points.

Gavrieli et al., with 33 participants, is likely to have been the most statistically powerful study, despite only performing each test once, and, as previously discussed, they observed an overall effect on glycaemic response to a low CHO dose. However, Louie et al. and Robertson et al. with 8 and 10 participants respectively, also observed an overall increase to the postprandial glycaemic response, so participant numbers alone have not determined the results in these cases. Interestingly, Gavrieli
et al. reported a greater effect in their overweight group and Robertson et al. recruited only overweight participants. As overweight people are more likely to be insulin-resistant this may have contributed to the observed effects; however, Robertson et al. reported their participants to have normal insulin sensitivity. Furthermore, Louie et al. studied normal-weight participants although, as previously discussed, they repeated their tests, thus increasing the power of their study to detect an effect.

Capillary blood sampling via the finger-prick technique is considered the gold standard in GI testing as it samples primarily arterial blood and therefore more accurately reflects glucose absorption. It has been demonstrated to be more sensitive to smaller differences in blood glucose than venous sampling and consistently gives a higher AUC than venous samples in GI testing \(^{(192)}\). Venous blood generally gives a lower glucose reading as some of the absorbed glucose will have been taken up by the tissues before it gets to the veins. Only seven of the twenty studies sampled capillary blood, however this does not explain why some studies observed an effect and others did not, as two of those that reported an overall effect sampled capillary blood and three took venous samples.

The doses of coffee given, whilst being physiological in terms of daily consumption, are generally higher than a person would normally consume in one drink. A typical mug of instant CC, as consumed in the UK, would contain approximately 2 g of instant coffee granules and would provide between 40 and 100 mg caffeine depending on the type of bean, as discussed in the introduction to this thesis. Only two studies \(^{(185,187)}\) gave what could be considered a normal dose of instant coffee but it is unclear from Young and Wolever how accurate their measurement was as they simply refer to serving a rounded teaspoon of instant CC \(^{(187)}\). For filter coffee, 1 level tablespoon (weighing 6-7 g) per cup of coffee is the normal measure. Using this as guidance, it would appear that two of the eight filter coffee studies \(^{(183,188)}\) gave a normal measure of filter coffee, with the others either higher or undeterminable. Dose does not appear to determine result however, as significant overall effects were observed for both low \(^{(185)}\) and high \(^{(184)}\) doses, nor was any overall dose-response effect observed in the three studies that compared different doses \(^{(183-185)}\), although Gavrieli et al. observed a dose-response effect in their overweight/obese subgroup \(^{(184)}\).

The volume of liquid consumed can affect gastric emptying, and therefore glucose response, however the differing volumes (200 - 812 ml) given in these studies do not appear to have determined the results, as increased glucose responses were reported for volumes at both ends of the range. Furthermore, no overall difference in glycaemic
response was observed when volumes of water ranging from 50 to 1000 ml were compared (187).

The majority of studies recruited healthy normal-weight participants. Of the two studies that measured glucose response in T2DM, one observed a trend for an increase in the overall response following CC ingestion (110), with CC resulting in a 4% greater AUC than control (p=0.06), whilst the other reported no difference (181). This difference in results may be explained by the differing methodologies employed as the participants in Alkaabi et al.’s study were instructed to take their diabetes medication before the study day, whilst those in Krebs et al.’s study were instructed to defer their medication until after the test. It should be noted, however, that the participants in both studies were relatively well-controlled by diet or oral hypoglycaemics, with mean HbA1c of 52 and 49 mmol/mol and mean fasted glucose of 7.5 and 6.4 mmol/L for Krebs et al. and Alkaabi et al. respectively. If the participants had exhibited poorer glycaemic control they may have observed a larger difference between treatments.

It has been demonstrated that 14 days of caffeine consumption, in caffeine-naïve individuals, results in partial but not complete acclimatisation to the acute effects caffeine has on glucose tolerance (193). It is advisable, therefore, to avoid using caffeine-naïve participants in acute studies in order to avoid exaggerated responses to caffeine exposure. Half of the studies in this review did not specify whether their participants were habitual coffee drinkers, which is a major confounder. Of the studies that provided this information, the majority reported regular coffee consumption in their participants.

Twelve studies included other treatments alongside CC and DC. Only the results related to coffee are covered in this review, however it should be noted that when multiple comparisons are made and post hoc adjustments made as a result of this, the statistical power will be reduced. Post hoc adjustments were made in nine of these studies, with 2/9 reporting an overall effect of coffee (184,185). It is possible that more significant effects would have been reported had a simpler study design been implemented. However, of the three studies that did not adjust statistically for multiple comparisons, two did not observe an overall effect (144,146). The remaining study compared five different drinks with a control and observed an overall effect on the postprandial glucose response (9). However, they reported a p-value of 0.022 for the comparison between CC and control; if this had been adjusted to account for the other tests, statistical significance would have been lost.
3.6 Conclusion

In summary, nearly 70% of acute studies reported a degree of increase in postprandial glucose response following CC ingestion, although the majority of these only observed a difference at individual time-points. Conversely, most studies reported no effect of DC on the postprandial glucose and insulin responses. It seems likely that CC does therefore have a detrimental effect on acute glycaemic control. What is unclear is whether a small overall increase or a small increase in peak values is physiologically and/or clinically relevant.

Whilst there have been a relatively large number of studies looking at these acute effects, the majority have been in a young, normal-weight, healthy population. There is clearly a need for more studies examining the effects of coffee on those with T2DM, particularly in those with poor glycaemic control. Studies in less healthy populations, such as those with MS or who are overweight/obese would also be advisable.

Finally, more research into the effects of normal doses of coffee would be beneficial. Instant coffee is the most commonly consumed coffee in the UK, yet only two of these twenty studies investigated the effects of a single cup of instant coffee.
Chapter 4. Dose-response study on the acute effects of coffee on glucose response

4.1 Introduction

Acute studies into the effects of CC on the postprandial glycaemic response have frequently reported a detrimental effect, with CC producing a greater postprandial glycaemic response than control \(^7,109,110\). Caffeine has also been demonstrated to produce greater glucose and insulin responses than control \(^125,126\), with some observing a larger effect from caffeine than CC \(^10\). This has led to the hypothesis that bioactive components in coffee, other than caffeine, may be attenuating the documented acute negative effects of caffeine on glycaemia. Chlorogenic acids and their metabolites have been suggested as possible candidates with studies involving isolated CGAs \(^144\) and coffee enriched with CGA \(^146\) both demonstrating a reduction in glucose response versus control, however a significant confounder with testing this hypothesis is that the majority of acute studies to date have not reported the CGA content of their coffees.

Previous studies have used large doses of CC, typically providing 3 - 6 mg caffeine per kg BW \(^126,161\). This would be the equivalent of 2.5 – 5 standard-sized (260 ml) servings, taken as a single dose, for a 70 kg person, based on the median caffeine content of instant coffee (as discussed in Chapter 1). Only one previous study, to our knowledge, has investigated the glycaemic effects of a single “normal” serving of instant coffee \(^187\), however there were several methodological concerns with that study which will be discussed later in this chapter.

4.2 Aims

This study was carried out in two parts:

Part A was a dose-response study with the lowest dose set at a single serving of CC. The aims were to investigate whether there was an acute effect of a single “normal” dose of CC on the postprandial glucose and insulin responses and to further investigate whether there was any effect of increasing doses.

Part B was a similar dose-response study where the CGAs and other coffee components were increased step-wise, as in Part A, whilst the caffeine dose remained constant. The aims of this part were to investigate the acute effects of increasing amounts of these non-caffeine coffee components.
4.3 Methods

4.3.1 Participants

Ten participants were recruited from the staff and student population of the University of Surrey by word of mouth, poster advertisement and internal emails. Written informed consent was obtained from all participants. Recruitment and data collection were carried out between August 2012 and February 2013. The study was given a favourable ethical opinion by the University of Surrey Ethics Committee (EC/2012/45/FHMS).

Participants were required to be overweight (BMI > 25 kg/m²), but otherwise healthy adult males, aged over 18 y, taking no prescription medication. They had to have been weight stable for the previous three months and to have no history of heart disease, diabetes, liver disease or any gastrointestinal or endocrine disorders. Smokers were excluded from participation in the study. Additionally, potential participants had to be habitual coffee drinkers (>= 4 servings per week).

4.3.2 Study design

The study was an eight-way randomised double-blind crossover study investigating the acute effects of different doses of DC with added caffeine on postprandial glucose and insulin response. Rather than using off-the-shelf CC, caffeine was added to DC to allow the amounts of caffeine and non-caffeine components to be varied independently in each drink.

The study was split into two parts. In Part A, three different doses of DC, with caffeine added in proportion to the DC, were compared with a control (water). This provided the equivalent of one, two and four servings of regular CC and allowed examination of the effects of escalating doses of CC. In Part B, the same three doses of DC were used, but this time each drink, including the control, contained the lowest amount of caffeine from Part A (100 mg). This allowed examination of the effects of escalating doses of the non-caffeine coffee components. The relative proportions of DC to caffeine for each drink are provided in Figure 4.3.2-1. The composition of each individual drink is detailed in Section 4.3.4.
4.3.3 General protocol

Potential participants were invited to attend a screening session (detailed in Chapter 2) where the study was explained to them in detail. If they fulfilled the inclusion criteria and decided to take part in the study, a date was set for their first study day. Each participant attended eight study days lasting approximately three hours each time.

For two days prior to each of the study days, participants were asked to refrain from exercise, alcohol and all coffee- and caffeine-containing food and drinks. They were asked to maintain the same diet the day before each visit and were given a standardised meal to consume the evening before. The standardised meal was a macaroni cheese ready meal (Tesco, Italian range, 450 g) providing 784 kcal, 31.2 g protein, 35.2 g fat, 80.5 g CHO and 10.1 g fibre.

After a 12 h overnight fast, participants arrived at the CIU between 8:00 and 9:00 am. They provided a baseline fasted capillary blood sample via the finger-prick technique. They then consumed the test drink and provided further capillary blood samples every 15 min for the next two hours. They were asked to rate each drink using a scale of 1 - 5 for each of four measures: strength, palatability, taste and smell (Appendix 5).

Figure 4.3.2.1 Diagrammatic representation of the relative proportions of decaffeinated coffee to caffeine for the test drinks.
In Part A, caffeine rises in proportion to decaffeinated coffee, whereas in Part B caffeine remains constant at 100 mg. DC: decaffeinated coffee.
The participants were permitted to bring work/books/laptops with them but were required to minimise physical activity and to remain in the CIU for the duration of the study day. Study days were separated by a washout period of at least five days and participants were given £200 on completion of the trial, as compensation for their time.

There were eight different test drinks with participants receiving each of the drinks over the course of the eight study days. A random sequence generator (https://www.random.org/sequences/) was used to generate the order for each participant. All treatments were double-blind; participants were simply advised that they were testing different coffees and were not told that they contained differing amounts of DC and caffeine. It was not possible to fully blind researchers and participants to the control drinks, however participants were not informed that there was any difference between the two control drinks and researchers were not aware which control drinks they were providing on each occasion.

4.3.4 Drink composition

Three different doses of DC were used (2, 4 and 8 g of instant DC granules), equivalent to that found in 1, 2 and 4 servings of coffee. In Part A, appropriate doses of caffeine were added to reflect the amount of caffeine found in the same doses of regular CC. As the DC was reported as containing 0.3% caffeine the amount of added caffeine was adjusted so that the total caffeine in each drink would be 100, 200 and 400 mg respectively. In Part B, the same three doses of DC were used, but caffeine was added such that they each had a total caffeine content of 100 mg. Detailed analysis of the DC, performed after the completion of the study, revealed the actual caffeine content to be 160 mg/100 g, resulting in all drinks having slightly less total caffeine than their target amounts. For reasons of clarity, the target caffeine content of each drink, rather than the actual caffeine content, will be referred to for the remainder of this thesis. The instant coffee granules were a commercially available DC, Kenco Decaff (The Kenco Coffee Company, Uxbridge, UK). The full coffee analysis can be found in Appendix 6.

All drinks were made by dissolving the DC and caffeine, along with 50 g glucose, in 200 ml boiling water. An additional 200 ml cold water was added to allow quick consumption. The different drink combinations along with their total CQA content are summarised in Table 4.3.4-1.
4.3.5 Blood collection

All blood samples were taken by finger prick with the use of a lancing device and collected into heparin fluoride microvette tubes (Sarstedt, Leicester, UK). These were refrigerated until the end of the study day then centrifuged and the plasma transferred into serum microvette tubes (Sarstedt, Leicester, UK). Plasma glucose concentrations were then immediately measured using the YSI 2300 STAT Plus™ (YSI Life Sciences, UK). Following glucose analysis the plasma was frozen at -20°C until the end of the study for batch analysis of insulin. Plasma insulin was measured by ELISA as described in Chapter 2.

4.3.6 Statistical analysis

All statistical analysis was carried out using SPSS Statistics software, version 22. The Shapiro-Wilks test was used to check normality of data. Overall treatment effects were analysed by RM ANOVA. A Bonferroni adjustment for multiple comparisons was applied when post hoc analysis was used. Statistical significance was taken as $p < 0.05$. A sample size of 10 was chosen in line with that recommended for GI testing, however a post hoc power calculation revealed the study to be underpowered to detect a difference in insulin. In excess of 23 participants would be required in order for the detected differences in insulin to be statistically significant at 80% power.

Table 4.3.4-1 Test drink composition.

<table>
<thead>
<tr>
<th>Drink number</th>
<th>Instant DC (g)</th>
<th>Target total caffeine (mg)</th>
<th>Actual total caffeine (mg)</th>
<th>Total CQA (mg)</th>
<th>Glucose (g)</th>
<th>Water (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Part A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>50</td>
<td>400</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>100</td>
<td>97</td>
<td>47</td>
<td>50</td>
<td>400</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>200</td>
<td>194</td>
<td>94</td>
<td>50</td>
<td>400</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>400</td>
<td>389</td>
<td>188</td>
<td>50</td>
<td>400</td>
</tr>
<tr>
<td>Part B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>50</td>
<td>400</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>100</td>
<td>97</td>
<td>47</td>
<td>50</td>
<td>400</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
<td>100</td>
<td>94</td>
<td>94</td>
<td>50</td>
<td>400</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>100</td>
<td>89</td>
<td>188</td>
<td>50</td>
<td>400</td>
</tr>
</tbody>
</table>

DC and caffeine powder were dissolved, along with 50 g glucose, in 200 ml boiling water to which 200 ml cold water was added to allow quick consumption. CQA: caffeoylquinic acid; DC: decaffeinated coffee.
4.4 Results

4.4.1 Baseline characteristics

The participants were overweight males (n=10) with a mean age of 30 y (SD 14) and mean BMI of 27.8 kgm\(^{-2}\) (SD 2.2). All were habitual coffee drinkers with a mean daily CC intake of 2.3 servings (range: 0.7 – 5.5) and a mean daily caffeine intake of 323 mg (range: 61 – 690 mg). Baseline characteristics are reported in Table 4.4.1-1. Fasted glucose, insulin, HOMA2_%B and HOMA2_%S are reported as the mean values for all visits.

Table 4.4.1-1 Baseline participant characteristics.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean (n=10)</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>30 14</td>
<td></td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.78 0.09</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>88.5 11.9</td>
<td></td>
</tr>
<tr>
<td>BMI (kgm(^{-2}))</td>
<td>27.8 2.2</td>
<td></td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>23.6 5.4</td>
<td></td>
</tr>
<tr>
<td>Fasted plasma glucose (mmol/L)</td>
<td>5.2 0.5</td>
<td></td>
</tr>
<tr>
<td>Fasted plasma insulin (pmol/L)</td>
<td>20 11</td>
<td></td>
</tr>
<tr>
<td>HOMA2_%B</td>
<td>48 18</td>
<td></td>
</tr>
<tr>
<td>HOMA2_%S</td>
<td>356 363</td>
<td></td>
</tr>
<tr>
<td>Daily CC intake (servings/day)</td>
<td>2.3 1.6</td>
<td></td>
</tr>
<tr>
<td>Daily caffeine intake (mg/day)</td>
<td>323 205</td>
<td></td>
</tr>
</tbody>
</table>

BMI: body mass index; CC: caffeinated coffee; HOMA: Homeostatic model of assessment; %S: sensitivity; %B: beta cell function.

4.4.2 Glucose and insulin

The majority of the glucose time-point data, including fasted and peak values, and all IAUC data were normally distributed. Log transformation of the time-points did not result in all data being normally distributed. As 88% of time-points were normally distributed, parametric tests were carried out on the original glucose dataset.

Half of all insulin time-point data were not normally distributed; log transformation resulted in normal distribution of the majority (85%), therefore parametric tests were carried out on the log-transformed insulin time-point data. Measures of insulin sensitivity and beta cell function were calculated as described in Chapter 2. The appropriate non-parametric and parametric tests were carried out on these measures. The HOMA2 calculator specifies acceptable ranges for steady state glucose (3.0 to 25.0 mmol/L) and specific insulin (20 to 300 pmol/L). Fasted glucose values in this
study all fell within the specified range, but insulin did not, with nearly half of all fasted insulin measures being less than 20 pmol/L. This was not unexpected as capillary sampling is known to result in lower insulin measures than venous (194). All values were included in the analysis as we were interested in relative values rather than absolute.

### 4.4.2.1 Escalating doses of decaffeinated coffee and caffeine

For Part A, where the amount of caffeine was varied in line with DC, there was a significant difference between treatments for glucose. Repeated measures ANOVA on the glucose time-points found a significant treatment effect (p=0.008) and a time * treatment effect (p=0.022), with the control drink displaying an earlier peak (15 min) than the other drinks (30 min). *Post hoc* analysis revealed a significant difference between the control drink and both the one and two serving equivalents (p<0.033). A similar result was observed for glucose IAUC, with an effect of treatment found (RM ANOVA, p=0.019) and *post hoc* analysis revealing a difference between the one serving equivalent and the control drink (p=0.008). When the glucose peak values were examined there was a significant difference between treatments (RM ANOVA, p=0.006), with *post hoc* analysis identifying a significant difference between the control and one serving equivalent (p=0.006) and a trend for a difference between the control drink and the two servings equivalent (p=0.056).

There were no significant differences between treatments for insulin (p>0.391), nor were there any significant differences in any insulin sensitivity and beta cell function measures (p>0.563). The insulin sensitivity and beta cell function measures are reported in **Table 4.4.2.1-1** and the two hour postprandial glucose and insulin responses are shown in **Figure 4.4.2.1-1**.

<table>
<thead>
<tr>
<th>Drink Code</th>
<th>Composition</th>
<th>HOMA2_%B</th>
<th>HOMA2_%S</th>
<th>Matsuda Index</th>
<th>AUCI/G ratio</th>
<th>Matsuda * AUGI/G</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0 0</td>
<td>48 14</td>
<td>278 112</td>
<td>21 14</td>
<td>12.6 7.3</td>
<td>198 57</td>
</tr>
<tr>
<td>2</td>
<td>2 100</td>
<td>46 11</td>
<td>305 177</td>
<td>40 74</td>
<td>13.9 8.2</td>
<td>324 408</td>
</tr>
<tr>
<td>3</td>
<td>4 200</td>
<td>44 19</td>
<td>408 359</td>
<td>36 41</td>
<td>11.5 6.3</td>
<td>296 374</td>
</tr>
<tr>
<td>4</td>
<td>8 400</td>
<td>52 22</td>
<td>399 572</td>
<td>18 16</td>
<td>14.3 7.5</td>
<td>202 106</td>
</tr>
</tbody>
</table>

*There were no significant differences between drinks for any measure (RM ANOVA, p>0.563); n=10. CF: caffeine; DC: decaffeinated coffee; HOMA: Homeostatic model of assessment; %S: sensitivity; %B: beta cell function; AUCI/G: ratio of insulin to glucose area under curve; Matsuda * AUGI/G: product of Matsuda index and AUCI/G ratio.*
Figure 4.4.2.1-1 Postprandial glucose and insulin responses to a control drink and three escalating doses of decaffeinated coffee and caffeine, equivalent to 1, 2 and 4 servings of standard caffeinated coffee. Significant differences between treatments were observed for glucose time-points (RM ANOVA, p=0.008) and peak values (p=0.006), with no difference between treatments for insulin. Error bars are SEM (n=10). CF: caffeine; DC: decaffeinated coffee.
4.4.2.2 Escalating doses of decaffeinated coffee, all with 100 mg caffeine

For Part B of the study, where all drinks contained 100 mg caffeine, there were no significant differences in postprandial glucose and insulin responses between treatments, however there was a time * treatment effect for insulin (RM ANOVA, p=0.017). An overall difference in peak glucose values was also observed (RM ANOVA, p=0.049) with no significant differences found between individual treatments in post hoc analysis. The 2 h postprandial glucose and insulin responses for Part B are shown in Figure 4.4.2.2-1.

Figure 4.4.2.2-1 Postprandial glucose and insulin responses to a control drink and escalating doses of decaffeinated coffee, all with 100 mg added caffeine.
There was an overall difference in peak glucose values (RM ANOVA, p=0.049) and a time * treatment effect for insulin (RM ANOVA, p=0.017). Error bars are SEM (n=10). CF: caffeine; DC: decaffeinated coffee.
There were no significant differences in any insulin sensitivity and beta cell function measures \((p>0.111)\). Insulin sensitivity and beta cell function measures for Part B are reported in Table 4.4.2.2-1.

### Table 4.4.2.2-1 Insulin sensitivity and beta cell function measures for Part B.

<table>
<thead>
<tr>
<th>Drink Code</th>
<th>DC (g)</th>
<th>CF (mg)</th>
<th>Composition</th>
<th>HOMA2_%B Mean</th>
<th>HOMA2_%S Mean</th>
<th>Matsuda Index Mean</th>
<th>Matsuda * AUC(_{I/G}) Mean</th>
<th>Matsuda * AUG(_{I/G}) Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0</td>
<td>100</td>
<td>54</td>
<td>300</td>
<td>210</td>
<td>21</td>
<td>10.4</td>
<td>173</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>100</td>
<td>45</td>
<td>287</td>
<td>101</td>
<td>39</td>
<td>10.0</td>
<td>262</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
<td>100</td>
<td>40</td>
<td>630</td>
<td>679</td>
<td>50</td>
<td>10.0</td>
<td>327</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>100</td>
<td>55</td>
<td>253</td>
<td>185</td>
<td>74</td>
<td>11.6</td>
<td>201</td>
</tr>
</tbody>
</table>

There were no significant differences between drinks for any measure \((RM ANOVA, p>0.111); n=10\). CF: caffeine; DC: decaffeinated coffee; HOMA: Homeostatic model of assessment; %S: sensitivity; %B: beta cell function; AUC\(_{I/G}\): ratio of insulin to glucose area under curve; Matsuda * AUG\(_{I/G}\) : product of Matsuda index and AUC\(_{I/G}\) ratio.

#### 4.4.2.3 Comparison of control drinks with and without added caffeine

There was a difference in glucose IAUC between the two control drinks \((paired \ t test, p=0.045)\), with a higher IAUC observed in the control drink containing caffeine. The two hour postprandial glucose and insulin responses for each control drink are shown in Figure 4.4.2.3-1.

There were no significant differences in any insulin sensitivity and beta cell function measures between the two drinks \((p>0.153)\). Insulin sensitivity and beta cell function measures for the two control drinks are reported in Table 4.4.2.3-1.

### Table 4.4.2.3-1 Insulin sensitivity and beta cell function measures for control drinks with and without added caffeine.

<table>
<thead>
<tr>
<th>Drink Code</th>
<th>DC (g)</th>
<th>CF (mg)</th>
<th>Composition</th>
<th>HOMA2_%B Mean</th>
<th>HOMA2_%S Mean</th>
<th>Matsuda Index Mean</th>
<th>Matsuda * AUC(_{I/G}) Mean</th>
<th>Matsuda * AUG(_{I/G}) Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>48</td>
<td>278</td>
<td>112</td>
<td>21</td>
<td>12.6</td>
<td>198</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>100</td>
<td>54</td>
<td>300</td>
<td>210</td>
<td>21</td>
<td>10.4</td>
<td>173</td>
</tr>
</tbody>
</table>

There were no significant differences between drinks for any measure \((p>0.153); n=10\). CF: caffeine; DC: decaffeinated coffee; HOMA: Homeostatic model of assessment; %S: sensitivity; %B: beta cell function; AUC\(_{I/G}\): ratio of insulin to glucose area under curve; Matsuda * AUG\(_{I/G}\) : product of Matsuda index and AUC\(_{I/G}\) ratio.
4.4.3 Test drink organoleptic properties

Parts A and B were examined separately. As the data were ordinal rather than continuous, non-parametric tests were used. Friedman’s ANOVA was used to test for an overall difference for each quality measure. Where significant differences were found, post hoc pairwise comparisons (Wilcoxon Signed Rank) were used to establish the sources of any differences. For Part A, no overall differences were found however trends were observed for differences in palatability (p=0.084) and smell (p=0.092).

For Part B, an overall difference was found for palatability (p=0.003) and taste (p=0.002) with no significant differences between individual pairs after adjustment for multiple comparisons. An overall difference in strength (p<0.001) was detected with post hoc analysis revealing drink 8 to have been perceived as stronger than drink 5.

Figure 4.4.2.3.1 Postprandial glucose and insulin responses to control drinks with and without added caffeine. There was a difference in glucose IAUC between the two control drinks (paired t test, p=0.045). Error bars are SEM (n=10). CF: caffeine; DC: decaffeinated coffee.
(p=0.007) and a trend for a difference between drinks 8 and 6 (p=0.096). A difference in smell (p=0.001) was also found with post hoc analysis revealing a difference between drinks 5 and 6 (p=0.005).

Mean responses for each of the four drink quality measures, strength, palatability, taste and smell, are reported in Table 4.4.2-1.

### Table 4.4.2-1 Study test drinks: results of organoleptic qualities questionnaire.

<table>
<thead>
<tr>
<th>Drink Code</th>
<th>Composition</th>
<th>Palatability</th>
<th>Strength</th>
<th>Taste</th>
<th>Smell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Part A</td>
<td>DC (g) CF (mg) Mean SD</td>
<td>Mean SD</td>
<td>Mean SD</td>
<td>Mean SD</td>
<td>Mean SD</td>
</tr>
<tr>
<td>1</td>
<td>0 0 2.9 1.3</td>
<td>2.6 1.1</td>
<td>2.8 1.2</td>
<td>2.8 0.8</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2 100 3.9 0.6</td>
<td>2.7 0.7</td>
<td>3.6 1.0</td>
<td>3.2 0.6</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>4 200 3.7 0.8</td>
<td>3.3 0.7</td>
<td>3.6 1.0</td>
<td>3.6 0.8</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>8 400 3.0 1.2</td>
<td>3.3 0.9</td>
<td>2.9 1.4</td>
<td>3.5 0.8</td>
<td></td>
</tr>
<tr>
<td>Part B</td>
<td>DC (g) CF (mg) Mean SD</td>
<td>Mean SD</td>
<td>Mean SD</td>
<td>Mean SD</td>
<td>Mean SD</td>
</tr>
<tr>
<td>5</td>
<td>0 100 2.7 1.1</td>
<td>2.1 1.1</td>
<td>2.5 1.1</td>
<td>2.6 0.8</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>2 100 3.7 0.7</td>
<td>2.5 0.8</td>
<td>3.6 0.7</td>
<td>3.7 0.5</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>4 100 4.1 0.6</td>
<td>3.1 0.7</td>
<td>3.8 0.8</td>
<td>3.7 0.8</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>8 100 3.8 0.8</td>
<td>3.6 0.8</td>
<td>3.4 0.8</td>
<td>3.5 0.8</td>
<td></td>
</tr>
</tbody>
</table>

Participants rated all drinks on a scale of 1-5 for palatability, strength, taste and smell. Parts A and B were analysed separately. Mean values within a column with unlike superscript letters were significantly different (Wilcoxon Signed Rank, p<0.008); n=10. CF: caffeine; DC: decaffeinated coffee.

### 4.5 Discussion

4.5.1 Escalating doses of decaffeinated coffee and caffeine

A significant difference was observed between drinks for Part A of the study where the added caffeine escalated proportionately to the DC, with post hoc analysis revealing differences between both the one and two serving equivalents and the control drink for overall glucose response and glucose peak values.

This is consistent with the majority of studies in the systematic review (Chapter 3), where almost 70% reported an increased postprandial glucose response following ingestion of CC. Young and Wolever, the only other study to our knowledge to use a “normal” dose of instant CC, observed a smaller effect than that observed in the current study, reporting an increased effect of CC at two time-points only (187). Similarly to the current study, their CHO dose was 50 g, their coffee was given concurrently with their meal and they took capillary blood samples. However their participants were younger, with a healthy BMI, whereas those in the current study were all overweight. It is likely that overweight participants are more insulin-resistant and possibly more susceptible to the glucose-raising effects of CC. Furthermore, they gave their
participants a mixed meal, which is likely to have been absorbed more slowly than the glucose used in this study. The CC consumed in this study contained approximately 100 mg caffeine; whilst this is within the normal range for instant CC, it is towards the top end of that range, as discussed in Chapter 1. It is possible that the larger effect observed here is a result of a higher dose of caffeine, however as Young and Wolever did not report their caffeine content, this cannot be confirmed. They also did not report the exact amount of CC consumed in their study, instead reporting the dose to be a rounded teaspoon. If their CC was not weighed, it is likely that their dose may have varied considerably. Additionally their participants were permitted to add milk to their CC, further increasing the likelihood of confounding. The authors did not report what percentage of participants opted to add milk, but they did state that it was standardised between visits for those who did.

A difference was observed in the time taken to reach peak plasma glucose, with the control drink peaking at 15 min and the three test drinks peaking later, at 30 min. This is consistent with the hypothesis that coffee may exert a beneficial effect by delaying glucose absorption \(^{(140,147)}\). The mean peak plasma glucose concentration following the control drink was 8.25 mmol/L whereas the peak following the two serving equivalent was 9.62 mmol/L, a mean increase of 17\% (1.37 mmol/L) in peak values. The magnitude of this increase is consistent with two other studies in the systematic review which observed increases in peak glucose of approximately 1 mmol/L \(^{(147)}\) and 2 mmol/L \(^{(7)}\).

No dose-response effect of coffee on blood glucose or insulin was observed in this part of the study. This is in contrast to a recent study which reported a dose-response effect on both glucose and insulin when using caffeine alone \(^{(195)}\). That study gave their participants doses of 1, 3 and 5 mg/kg BW and reported linear increases in glucose AUC of 11.2 mmol/L.120 min and insulin AUC of 5.8\% for each 1 mg/kg increase in caffeine. The mean caffeine contents of the drinks in the current study were similar, with participants receiving mean caffeine doses of 1.15, 2.30 and 4.60 mg/kg, however no linear increase in postprandial glucose and insulin response was observed as a result. In contrast the greatest increase in glucose IAUC (equivalent to 49 mmol/L.120 min for each 1 mg/kg) was observed between the control drink and the lowest coffee dose (100 mg added caffeine) with a smaller increase between the one and two serving equivalents (17 mmol/L.120 min per mg/kg) and a decrease between the two and four serving equivalents (-13 mmol/L.120 min per mg/kg). For insulin IAUC, a 22\% increase was observed for each 1 mg/kg between control and the one serving equivalent, with
a decrease between the next two drinks (-15% per mg/kg) and a subsequent increase between the two highest doses (8% per mg/kg).

Gavrieli et al. compared two different amounts of instant CC, providing 3 and 6 mg/kg BW, to a control [184]. Although they calculated their doses per kg BW, the mean amounts of caffeine in their two CC doses, 228 mg and 457 mg, were similar to the two higher amounts of caffeine used in this study (200 mg and 400 mg, in drinks 3 and 4 respectively). However, the mean amounts of instant CC they gave their participants, 6.0 g and 11.9 g, were 50% higher than the two highest doses (4 and 8 g) in the current study, so it is likely that their total CGA content was higher. They reported a higher glucose IAUC in both coffee doses compared with control and also observed a time * treatment effect for glucose and insulin responses. In line with this study, they observed no significant overall dose-response effect, despite having a higher number of participants (n=33). Interestingly they reported a greater effect in their female and their overweight participants, which cannot be explained by body size as doses were proportional to bodyweight. Furthermore when they performed their analysis on the overweight/obese subset of their participants they did observe a dose-response effect on glucose IAUC. Their overweight/obese participants were of a similar age, weight and BMI to participants in the current study, so a difference in baseline characteristics cannot explain the difference in results between the studies. The lack of a dose-response effect in the current study may however be due to high inter- and intra-individual variation in glycaemic response, which was apparent when individuals’ responses were examined and is in line with that previously demonstrated [190]. A larger sample size may have had sufficient power to detect a dose-response effect, particularly when one considers that the two studies to observe such an effect had higher numbers, with Gavrieli et al. recruiting 17 participants to their overweight/obese group and Beaudoin et al. recruiting 24 participants to their caffeine study.

Surprisingly, post hoc analysis found the increased glucose response only applied to the one and two serving equivalents and not to the highest dose. It is unlikely that a greater insulin response to the high caffeine levels had suppressed the glucose response in this case, as no significant differences between drinks for the insulin response were found. Furthermore, although Beaudoin et al. reported a dose-response effect on insulin, there was no resultant suppression of glucose [195].

4.5.2 Escalating doses of decaffeinated coffee, all with 100 mg caffeine

There was no overall treatment effect on either postprandial glucose or insulin for Part B of the study, where all drinks, including the control, contained 100 mg caffeine. There
was however an overall difference in glucose peak values and a time * treatment effect for insulin, with no significant differences between individual pairs in post hoc comparisons.

These findings suggest that a relatively small dose of caffeine, 100 mg, may be sufficient to override any potential beneficial effects of increasing amounts of other coffee compounds. However it should be noted, as discussed previously, that this amount of caffeine is towards the upper end of the range typically found in a single serving of CC. Furthermore, the total amount of CGAs in the coffee, 29.1 mg/g (23.5 mg/g CQA), equivalent to 58.1 mg (47 mg CQA) in the lowest dose, is relatively low in comparison to the amounts found in brewed (27 – 94 mg CQA/serving) and espresso (24 – 422 mg CGA/serving) coffees, as discussed in the introduction to this thesis, although it is comparable to typical amounts found in instant DC.

Whilst there was no dose-response effect of increasing the non-caffeine components of coffee, a non-significant reduction in glucose IAUC with each increasing dose was observed, unlike in Part A. However, it is inadvisable to form any conclusions based on these non-significant differences, particularly when one considers that whilst the lowest mean glucose IAUC (207 mmol/L.120 min) was observed following the highest dose of DC, the actual IAUC value was very similar to the mean IAUC observed in the control drink (210 mmol/L.120 min).

### 4.5.3 Comparison of control drinks with and without added caffeine

Whilst no overall difference was observed between the two control drinks when the time-point data were analysed, the control drink with added caffeine (Part B) produced a significantly higher glucose IAUC than the control without caffeine (Part A). This result is in line with others who have compared the effects of caffeine on the postprandial glucose response \(^{10,125}\). Unlike several others \(^{10,125,196}\) there was no increased insulin response to the caffeine in the current study, however these other studies gave higher doses of caffeine (typically 5 mg/kg). As noted previously, Beaudoin et al. observed a dose-response effect on insulin with caffeine doses starting at 1 mg/kg, however their study had 24 participants and therefore greater power to observe an effect at smaller doses \(^{195}\).

### 4.5.4 Test drink organoleptic properties

For Part A, no significant differences were found for any measure however trends were observed for differences in palatability and smell. For Part B, overall differences were
found for palatability and taste with no significant pairwise differences. An overall difference in strength was also detected with *post hoc* analysis revealing the highest dose CC to have been perceived as stronger than the control and a trend for a difference between the highest and lowest doses of CC. A difference in smell was also found with *post hoc* analysis revealing a difference between the control and the lowest dose CC.

When individual responses were examined, there was a large amount of inter-individual variation on each of the ratings. Ratings of palatability in particular varied with scores for the highest dose spanning the entire range from completely unpalatable (1) to extremely palatable (5), depending on whether the individual preferred strong or weak coffee. A similar range of responses was observed for the control drinks, corresponding to how much the participant liked sweet drinks. Interestingly, the participants were better able to detect differences in coffee strength in Part B, where the amount of caffeine in each drink was 100 mg, although only a trend was observed between highest and lowest doses. Many participants commented that the sweetness of the drinks overrode other factors and made it difficult to rate the drinks. It should also be noted that participants did not have the opportunity to directly compare individual drinks as each drink was rated on the study day and greater variation in response can be expected when rated on different days.

An obvious difference between the control and coffee drinks is the presence or absence of coffee with its characteristic bitter taste. As the hedonic properties of food/drink have been demonstrated to impact acutely on postprandial nutrient handling (197), it was important to exclude this as a simple pre-ingestive mechanism contributing to any observed differences. There were no significant differences in Part A for either palatability or taste which would indicate that the impact of the coffee on glucose metabolism was due to a true post-ingestive mechanism. However, there was a slight trend for an overall difference in palatability and examination of the data revealed ratings for both palatability and taste dropped non-significantly with the highest dose coffee, such that the mean ratings for this dose were very similar to the control ratings. This may therefore have made some contribution to the lack of observed effect of the highest coffee dose. However, this does not explain why other studies have demonstrated an increased postprandial glucose response following doses similar to the highest dose in the current study (8,126).
4.5.5 Limitations/confounders

Doses were not adjusted according to participants’ bodyweights in contrast to several previously discussed studies (7,109,110,184,195) in order to reflect normal nutritional intake patterns. When the caffeine content was converted to mg/kg BW the lowest dose (100 mg caffeine) gave a mean value of 1.15 mg/kg (range 0.9 – 1.5 mg/kg) and the highest dose (400 mg caffeine) was 4.6 mg/kg (range 3.7 – 6.1 mg/kg). This variation in dose between participants may be a confounder in determining a dose-response relationship. To examine this, a post hoc analysis was performed on the glucose IAUC data from Part A. Firstly the data was re-analysed with the three participants with the smallest and largest bodyweights omitted (equivalent to 0.9, 1.4 and 1.5 mg/kg at the lowest coffee dose), leaving seven participants (range 1.0-1.2 mg/kg). The previously observed overall difference in IAUC remained, but with reduced significance (p=0.041) and no significant pairwise differences. The analysis was then repeated with all 10 participants categorised into high or low bodyweight, which was added into the analysis as a cofactor. Again, an overall difference in glucose IAUC between drinks was observed (p=0.028), but with no difference between the high and low group and no interaction effect. These results suggest that the dosing methodology did not impact on the results.

Another possible confounder may be different genetic polymorphisms in the CYP1A2 gene which have been demonstrated to affect the rate of caffeine metabolism in humans (198), as discussed in Section 1.4.5. Unfortunately the participants were not genotyped for these polymorphisms as this may have partly explained the high inter-individual variations observed.

As previously discussed, the relatively low number of participants may be insufficient to determine a dose-response relationship. Furthermore, a post hoc power calculation detailed in Section 4.3.6 revealed the study to be underpowered to detect a difference in insulin.

As noted earlier in this chapter (Section 4.3.4), independent analysis of the instant DC, performed after the conclusion of the study, revealed the caffeine content to be approximately half that originally reported by the suppliers. This resulted in the highest doses having 11 mg less caffeine than their target amounts. This equates to an 11% reduction in target amount for the highest dose in Part B, but only a 3% reduction for the highest dose in Part A. Whilst this is not ideal, it does not appear to have impacted on the results. It is unlikely that the unexpected lack of effect on the postprandial glucose response, of the highest dose in Part A, was caused by the reduced caffeine.
content, as the absolute caffeine content was higher than that of the two lower doses, which did increase the glucose IAUC.

The ratio of caffeine to total CGA in the study was not ideal. If one considers that a typical 2 g serving of instant CC contains 41 - 85 mg CGA and 50 - 100 mg caffeine, then it becomes apparent that the lowest dose was disproportionately high in caffeine. Whilst quite feasible, 100 mg caffeine was at the high end of the normal range, whereas the total CGA content (58 mg) was in the low-mid range.

A large amount of inter-individual variation was observed in the postprandial glucose response to the test drinks. This could perhaps have been reduced if a starchy meal had been provided along with the test drinks, rather than glucose, as OGTTs have been demonstrated to result in CVs 2 - 3 times higher than white bread (199). However, the use of glucose in this study had some benefit, in that it served to somewhat disguise the taste and strength of the coffees as previously discussed.

### 4.6 Conclusion

In support of the hypothesis, this study has demonstrated that a single serving of instant CC, as typically consumed in the UK, is sufficient to disrupt postprandial glucose metabolism. It is not known however, whether the observed differences, whilst statistically significant, are physiologically relevant. The participants were healthy, albeit overweight, insulin-sensitive individuals with normal glucose tolerance and the observed increase in postprandial glucose response following CC ingestion was quickly resolved in this group. It is not known how those with IFG or T2DM would respond to a similar intervention.

In Part B of the study, there was no beneficial effect of increasing doses of non-caffeine components on postprandial glucose and insulin metabolism when each drink contained 100 mg caffeine. This is in contrast to the literature demonstrating beneficial effects of CGAs and suggests that the amount of caffeine in a single serving of CC may attenuate any possible beneficial effects of these other coffee components. However, as discussed, the drinks were relatively low in total CGA content and 100 mg caffeine is a relatively large dose for a single serving. It is possible an effect may have been observed if the balance of caffeine to CGA was altered. It could also be argued that caffeine should not have been included in Part B of the study. However, whilst the aim was to investigate the effects of escalating doses of the non-caffeine components it was decided to do this within the framework of a caffeinated drink as most people in the UK who drink coffee do not drink DC.
Whilst these results, and others previously discussed, demonstrate that an acute dose of CC can temporarily disrupt postprandial glucose metabolism, it should be noted that the majority of these investigations have been carried out in healthy people. It is important that further studies are carried out in those with IFG and T2DM where the effects of coffee may be clinically relevant.

It should also be noted that the results of acute studies cannot be simply translated into chronic effects. Longer-term interventions investigating these chronic effects are now needed as the current evidence for a beneficial effect of coffee in terms of reduced risk of diseases such as T2DM is based on cohort studies which cannot prove a causal relationship.

This work has been published in the British Journal of Nutrition:

Chapter 5. The acute effects of repeated doses of decaffeinated coffee on the postprandial glucose response

5.1 Introduction

As discussed in the introduction to this thesis, coffee contains several bioactive compounds, such as CGAs and their derivatives, which may attenuate some of the acute glucose-raising effects of caffeine. Indeed, studies involving CGA alone (144) and coffee enriched with CGA (146) have demonstrated a reduction in glucose AUC compared to a control. It would seem reasonable that DC might produce a similar effect. Studies investigating the effect of DC however have displayed mixed results with some finding no effect on postprandial glucose (144,146), some reporting a reduced glucose IAUC (10) and some observing higher glucose at several time-points (126) or an increase in IAUC (8). One limitation of these previous studies is the high doses of DC given to participants, with no studies, to our knowledge, investigating the effects of a single serving.

The postprandial glycaemic response has been observed to be greater in the afternoon and evening than in the morning (200–202). This is likely due to circadian rhythms in both insulin (203) and cortisol (204) production, as cortisol pulses have been observed following lunch and evening meals (205) and higher insulin has been observed in the morning (202). It has been hypothesised that the timing of coffee intake may modulate the preventative effect of coffee on T2DM risk suggested by the epidemiology; a prospective cohort study of almost 70,000 women reported an association between both CC and DC and reduced risk of T2DM, but only if the coffee was consumed at lunchtime (91). It is possible that the acute effects of coffee on postprandial glycaemia may also vary at different times of day. Whilst CC and, to a lesser extent, DC consumed with a morning meal have been demonstrated to produce a higher postprandial glucose and insulin response to a later meal (8), no studies to our knowledge have examined the effects of repeated dosing or the effects of DC consumed at lunchtime on the postprandial glycaemic response.
Chapter 5.

5.2 Aims

The aims of this study were:

- To investigate whether a single serving of DC has an effect on postprandial blood glucose and insulin when taken with a mixed meal in the morning.
- To investigate whether a single serving of DC has an effect on postprandial blood glucose and insulin when taken with a mixed meal in the afternoon (second meal of the day).
- To investigate whether a single serving of DC taken with a mixed meal in the morning has a carry-over effect on the second meal postprandial glucose and insulin responses.
- To investigate whether there is any effect of repeated doses of DC on the postprandial glucose and insulin responses.

5.3 Methods

This study was carried out with the assistance of two undergraduate students, Eithne McGonigle and Olivia Chesterman, as their final year project.

5.3.1 Participants

Participants were recruited from the student population at the University of Surrey by word of mouth, poster advertisement and internal emails. Written informed consent was obtained from all participants. The study was given a favourable ethical opinion by the University of Surrey Ethics Committee (EC/2012/110/FHMS). Recruitment and data collection were carried out between December 2012 and March 2013.

Participants were required to be healthy adults, aged 18 - 40 y, taking no prescription medication other than oral contraceptives. They were required to be weight stable and to have no history of heart disease, diabetes, liver disease or any gastrointestinal or endocrine disorders. They were ineligible if they were regular smokers. Women not using hormonal contraceptives were excluded from the study, as women display variability in their glycaemic response throughout the menstrual cycle with impaired glucose metabolism having been observed during the luteal phase (206). All participants were required to be habitual coffee drinkers, with a minimum intake of four cups per week. Ten participants were recruited to the study, consistent with that recommended for GI testing (173).
5.3.2 Study design

A four-way open-label randomised crossover study comparing the effects of DC with a control on the postprandial glycaemic response to a mixed meal at different times of day.

5.3.3 General protocol

Potential participants were invited to attend a screening session (detailed in Chapter 2) where the study was explained to them in detail. If they fulfilled the inclusion criteria, and decided to take part in the study, a date was set for their first study day.

Each participant attended four study days each lasting approximately 6 h. On each study day they consumed a standardised study meal along with a test drink for breakfast and lunch. Capillary blood samples were taken for 2 h following each meal. The detailed study day protocol can be found in Section 5.3.3.3.

There were four different drink combinations (Table 5.3.3-1) with participants receiving each of the combinations over the course of the four study days. A random sequence generator (https://www.random.org/sequences/) was used to generate the order for each participant. Study days were separated by a washout period of at least three days and participants were given £100 on completion of the trial, as compensation for their time.

<table>
<thead>
<tr>
<th>Combination</th>
<th>Test drink Breakfast</th>
<th>Test drink Lunch</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>Control</td>
</tr>
<tr>
<td>2</td>
<td>DC</td>
<td>Control</td>
</tr>
<tr>
<td>3</td>
<td>DC</td>
<td>DC</td>
</tr>
<tr>
<td>4</td>
<td>Control</td>
<td>DC</td>
</tr>
</tbody>
</table>

Table 5.3.3-1 Test drink combinations.

DC: decaffeinated coffee.

5.3.3.1 Test drink composition

The test drink was either DC or a control drink (water). The DC consisted of 2 g instant DC granules dissolved in 100 ml boiling water and made up to 200 ml with cold water to facilitate rapid consumption. It was sourced from the same batch of commercially available DC (Kenco Decaff) used in the dose-response study (Chapter 4). The control drink was 200 ml cold water.
5.3.3.2 Study meal composition

A standardised study meal consisting of cornflakes, milk, yoghurt and sugar was given, along with the test drink, for both breakfast and lunch on each study day. Details of the study meal are provided in Table 5.3.3.2-1.

Table 5.3.3.2-1 Standardised study meal composition.

<table>
<thead>
<tr>
<th>Meal items</th>
<th>Portion size (g)</th>
<th>Energy (kcal)</th>
<th>CHO (g)</th>
<th>Fat (g)</th>
<th>Protein (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kellogg's Corn Flakes®</td>
<td>50</td>
<td>189</td>
<td>42</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Semi-skimmed milk</td>
<td>225</td>
<td>113</td>
<td>11</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Sugar</td>
<td>8</td>
<td>32</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Activia® Pouring Yoghurt, vanilla</td>
<td>150</td>
<td>99</td>
<td>14</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>433</strong></td>
<td><strong>75</strong></td>
<td><strong>7</strong></td>
<td><strong>17</strong></td>
<td></td>
</tr>
</tbody>
</table>

CHO: carbohydrate.

5.3.3.3 Study day protocol

The timeline for the study day is provided in Figure 5.3.3.3-1.

For two days prior to each study day the participants were asked to refrain from exercise and all caffeine-containing food and drinks. On the day prior to each study day they were also asked to avoid alcohol. They were provided with a standardised meal to consume on the evening prior to each study day, a vegetarian lasagne (Tesco, Italian range, 450 g) providing 509 kcal, 14.5 g protein, 26.0 g fat, 51.9 g CHO and 4.8 g fibre.

After a 12 h overnight fast, participants arrived at the CIU between 8:00 and 9:00 am. They provided a baseline fasted capillary blood sample via the finger-prick technique. They then consumed their first test drink of the day along with the study meal and provided further capillary blood samples over the next 2 h (taken at 15, 30, 60, 90 and 120 min time-points from the start of the meal).
This process was then repeated for the lunchtime drink and meal, with the second baseline sample taken at 200 min, the meal taken at 215 min and subsequent samples at 230, 245, 275, 305 and 335 min time-points. Each study session lasted approximately six hours, during which time the participants were required to stay in the CIU. They were permitted to bring work/books/laptops with them but were required to minimise physical activity.

### 5.3.4 Blood Collection

All blood samples were taken by finger prick with the use of a lancing device and collected into heparin fluoride microvette tubes (Sarstedt, Leicester, UK). These were refrigerated until the end of the study day then centrifuged and the plasma transferred into serum microvette tubes (Sarstedt, Leicester, UK). Plasma glucose concentrations were then measured using the YSI 2300 STAT Plus™ (YSI Life Sciences, UK). Following glucose analysis, the plasma was frozen at -20 °C until the end of the study for subsequent batch analysis of insulin by ELISA, as described in Chapter 2.

### 5.3.5 Statistical analysis

All statistical analysis was carried out using SPSS Statistics software, version 22. The Shapiro-Wilks test was used to check normality of data. Paired t tests were used for comparisons between treatments when the data were normally distributed and the equivalent non-parametric tests were used when they were not. Time-point data were analysed by RM ANOVA. Statistical significance was taken as a p-value < 0.05.

A sample size of 10 was chosen in line with that recommended for GI testing, however a post hoc power calculation revealed the study to be underpowered to detect a difference in insulin. In order for the observed differences in insulin to be statistically significant at 80% power, would have required in excess of 34 participants (based on IAUC results from the repeated dosing comparison, Section 5.4.2.5).

The following combinations were compared to test the specific aims of the study:

- The means of combinations 1 and 4 (control first) and 2 and 3 (DC first) for the morning meal only, were compared to examine whether there was any effect of DC versus control on the morning meal glycaemic and insulinaemic responses.
- Combinations 1 (control/control) and 4 (control/DC) were compared to test for an effect of lunchtime coffee on the glycaemic response to the second meal.
• Combinations 1 (control/control) and 2 (DC/control) were compared to examine whether there was any carry-over effect of morning DC on the glycaemic response to a second meal.

• Combinations 1 (control/control), 2 (DC/control) and 3 (DC/DC) were compared for evidence of a repeated-dose effect.

5.4 Results

5.4.1 Baseline characteristics

Participants were healthy females (n=10) with a mean age of 21 y (SD 2) and BMI of 21.6 kg m⁻² (SD 2.1). Baseline characteristics are reported in Table 5.4.1-1. All were regular coffee drinkers with a mean weekly coffee intake (CC and DC combined) of 16 servings (range: 4 – 42) and a mean daily caffeine intake of 312 mg (range: 158 – 559 mg). Fasted glucose, insulin, HOMA2_%B and HOMA2_%S are reported as the mean values for all visits.

Table 5.4.1-1 Baseline participant characteristics.

<table>
<thead>
<tr>
<th>Mean (n=10)</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>21  2</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.65 0.06</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>58.8 7.6</td>
</tr>
<tr>
<td>BMI (kgm⁻²)</td>
<td>21.6 2.1</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>21.7 4.4</td>
</tr>
<tr>
<td>Fasted plasma glucose (mmol/L)</td>
<td>4.8 0.3</td>
</tr>
<tr>
<td>Fasted plasma insulin (pmol/L)</td>
<td>27 23</td>
</tr>
<tr>
<td>HOMA2_%B</td>
<td>67 25</td>
</tr>
<tr>
<td>HOMA2_%S</td>
<td>275 232</td>
</tr>
<tr>
<td>Daily CC intake (servings/day)</td>
<td>2.2 1.9</td>
</tr>
<tr>
<td>Daily caffeine intake (mg/day)</td>
<td>312 139</td>
</tr>
</tbody>
</table>

BMI: body mass index; CC: caffeinated coffee; HOMA: Homeostatic model of assessment; %S: sensitivity; %B: beta cell function.
5.4.2 Glucose and insulin

The majority of the glucose time-point data were normally distributed; log transformation of the time-points did not result in all data being normally distributed. As 44/48 time-points were normally distributed, parametric tests were carried out on the original dataset for glucose. The majority of the insulin time-point data (28/48) were not normally distributed; log transformation resulted in most (42/48) being normally distributed. Parametric tests were therefore carried out on the log-transformed insulin time-point data. Measures of insulin sensitivity and beta cell function were calculated as described in Chapter 2. None were normally distributed, either before or after log transformation; non-parametric tests were carried out on this data.

Glucose and insulin values at 200 min were used to calculate HOMA2_%B and HOMA2_%S for the afternoon session. Although the participants were not fully fasted at 200 min, this was the last measure taken prior to the second meal. Furthermore, glucose and insulin values had returned to fasted levels at this time-point for 3 of 4 treatments with no significant differences between 0 and 200 min measures for insulin or glucose for any treatment, but a trend for higher glucose (+0.15 mmol/L) at 200 min for treatment 4 (p=0.053). As in the dose-response study (Chapter 4), fasted glucose values all fell within the acceptable range for the HOMA2 calculator but insulin did not. All values were included in the analysis however, as we were interested in relative values rather than absolute.
5.4.2.1 Circadian rhythms in postprandial glucose and insulin response

Mean glucose values were higher 120 min after the second meal (335 min time-point) than 120 min after the first meal (RM ANOVA, p=0.002), indicating a time-of-day effect, however there was no difference in the change between treatments (treatment * time, p=0.823). There were no significant differences between pre-meal glucose values (0 and 200 min) for any treatment, but there was a trend for a difference for treatment 1 (control/control), where mean fasted glucose was 0.15 mmol/L higher at 200 min (paired t test, p=0.053). The postprandial glucose response across both meals for all treatments is shown in Figure 5.4.2.1-1.

![Figure 5.4.2.1-1](image)

**Figure 5.4.2.1-1** Postprandial glucose response over the course of two meals for each of four drink combinations.
Baseline blood samples were taken at -15 and 200 min followed by the standardised study meals which were taken, along with the study drinks, at 0 and 215 min. Glucose values were higher 120 min after the second meal than after the first (RM ANOVA, p=0.002). Error bars are SEM (n=10).
Similarly for insulin, mean values were higher 120 min after the second meal than after the first meal (RM ANOVA, p=0.003), with no difference in the change between treatments (treatment * time, p=0.868), indicating a time-of-day effect on insulin response. There were no significant differences between pre-meal insulin values (0 and 200 min) for any drink combination. The postprandial insulin response across both meals for all treatments is shown in Figure 5.4.2.1-2.

![Figure 5.4.2.1-2](image)

**Figure 5.4.2.1-2  Postprandial insulin response over the course of two meals for each of four drink combinations.**

Baseline blood samples were taken at -15 and 200 min followed by the standardised study meals which were taken, along with the study drinks, at 0 and 215 min. Insulin values were higher 120 min after the second meal than after the first (RM ANOVA, p=0.003). Error bars are SEM (n=10).
5.4.2.2 Effects of decaffeinated coffee at a morning meal

The means of time-points and IAUCs were calculated for treatments 1 and 4 (control at breakfast) and for 2 and 3 (DC at breakfast) in order to compare the effects of DC with control on the postprandial glucose and insulin response to the morning meal.

There were no significant differences between treatments for plasma glucose IAUC over either the first 120 or 200 min (paired t test, p>0.434), nor were there any differences when the time-points were compared over the same periods (RM ANOVA, p>0.352). The mean postprandial glucose response for each treatment is shown in Figure 5.4.2.2-1.

![Figure 5.4.2.2-1](image)

Figure 5.4.2.2-1 Postprandial glucose response to a mixed meal taken with either decaffeinated coffee or control.

There were no differences between treatments over the first 120 or 200 min for either IAUC (paired t test, p>0.434) or time-point data (RM ANOVA, p>0.352). DC: decaffeinated coffee. Error bars are SEM (n=10).

There were also no significant differences between treatments for plasma insulin IAUC over either the first 120 or 200 min (Wilcoxon Signed Rank, p>0.168), nor were there any differences when the time-points were compared over the same periods (RM ANOVA, p>0.445). The mean postprandial insulin response for each treatment is shown in Figure 5.4.2.2-2. There were no significant differences between DC and control for any of the insulin sensitivity and beta cell function parameters (Wilcoxon Signed Rank, p>0.138).
Figure 5.4.2.2-2  Postprandial insulin response to a mixed meal taken with either decaffeinated coffee or control.
There were no differences between treatments over the first 120 or 200 min for either IAUC (Wilcoxon Signed Rank, p>0.168) or time-point data (RM ANOVA, p>0.445). DC: decaffeinated coffee. Error bars are SEM (n=10).
5.4.2.3 Effect of decaffeinated coffee at lunchtime

There was no difference in either IAUC (Wilcoxon Signed rank test, p>0.138) or time-point data (RM ANOVA, p>0.371) between treatments 1 (control/control) and 4 (control/DC) for the postprandial glucose and insulin responses to the second meal. There was however an effect on HOMA2_%B (Wilcoxon Signed Rank, p=0.037), with higher values observed for DC at lunchtime (78.2, SD 36.0) than control (60.6, SD 21.3). There was also a trend for an effect on the AUC_{IG} ratio (Wilcoxon Signed Rank, p=0.074), with DC at lunchtime resulting in a higher AUC_{IG} ratio (20.9, SD 9.7) than control (18.5, SD 7.6). The postprandial glucose and insulin responses to the two treatments are shown in Figure 5.4.2.3-1.

Figure 5.4.2.3-1 Postprandial glucose and insulin responses to breakfast and lunch, when the lunchtime meal is accompanied by either decaffeinated coffee or control. There were no differences between treatments for either IAUC (p>0.138) or time-point data (p>0.371), for glucose (A) or insulin (B), following the second meal. DC: decaffeinated coffee. Error bars are SEM (n=10).
5.4.2.4 Effects of morning decaffeinated coffee on the postprandial response to a subsequent meal

There were no differences in either IAUC (glucose: paired t test, p=0.535; insulin: Wilcoxon Signed rank, p=0.799) or time-point data (RM ANOVA, p>0.673) or any insulin sensitivity or beta cell function measures (Wilcoxon Signed Rank, p>0.332), over the entire study day (0-335 min), between treatments 1 (control/control) and 2 (DC/control), nor were there any differences in any measure when the morning (p>0.113) and afternoon (p>0.284) meals were examined separately. The postprandial glucose and insulin responses to the two treatments are shown in Figure 5.4.2.4-1.

Figure 5.4.2.4-1 Postprandial glucose and insulin responses to breakfast and lunch, when breakfast is accompanied by either decaffeinated coffee or control. There were no differences between treatments for either IAUC (p>0.534) or time-point data (p>0.673), for glucose (A) or insulin (B), over the entire study day. DC: decaffeinated coffee. Error bars are SEM (n=10).
5.4.2.5 Effect of multiple doses of decaffeinated coffee

There was no difference between either IAUC (glucose: paired t test, \( p=0.396 \); insulin: Wilcoxon Signed rank, \( p=0.333 \)) or time-point data (RM ANOVA, \( p>0.153 \)) over the entire study day (0-335 min), between treatments 1 (control/control), and 3 (DC/DC) for glucose or insulin. Nor was there any difference in any measure when the results for the morning meals were compared (\( p>0.168 \)). There was however a trend for an effect on the insulin time-point data following the second meal (RM ANOVA, \( p=0.058 \)) and a trend for an effect on the second meal AUC_{IG} ratio (Wilcoxon Signed Rank, \( p=0.074 \)) with DC at breakfast and lunchtime resulting in a lower insulin response and a lower AUC_{IG} ratio than control. The postprandial glucose and insulin responses to the two treatments are shown in Figure 5.4.2.5-1.

**Figure 5.4.2.5-1** Postprandial glucose and insulin responses to breakfast and lunch, when both meals are accompanied by either decaffeinated coffee or control. There were no differences between treatments for either IAUC (\( p>0.332 \)) or time-point data (\( p>0.153 \)), for glucose (A) or insulin (B), over the entire study day. There was a trend for a lower insulin response to the afternoon meal following DC at both meals (\( p=0.058 \)). DC: decaffeinated coffee. Error bars are SEM (n=10).
When one (treatment 2) and two doses (treatment 3) of DC were compared with control (treatment 1), there was no difference in either IAUC or time-point data (RM ANOVA, \( p > 0.482 \)) over the entire study day (0-335 min) for glucose or insulin. Nor were there any differences in insulin sensitivity or beta cell function measures between the three treatments (Friedman’s ANOVA, \( p > 0.272 \)).

5.5 Discussion

5.5.1 Circadian rhythms in postprandial glucose response

A time-of-day effect, consistent with the literature, was observed with glucose and insulin levels being higher 120 min after the second meal than after the first. Glucose and insulin levels at 120 min were also higher than the fasted levels for all treatments which is perhaps surprising, given the study was carried out in young healthy women, however in most cases they had returned to fasted levels immediately prior to the second meal.

5.5.2 Effect of decaffeinated coffee at morning meal

There were no significant differences between DC and control for glucose or insulin over either the first 120 or 200 min, nor were there any differences for the insulin sensitivity and beta cell function measures. This lack of an effect of a single dose of DC on postprandial glucose and insulin response is in line with the majority of studies from the systematic review (Chapter 3), where 8/11 found no effect on glucose and 7/9 found no effect on insulin.

Of the studies that found an effect of DC on glucose, one observed a lower IAUC following DC in comparison with a control \(^{10} \), one observed a greater glucose IAUC \(^{8} \), and one reported higher glucose values at two time-points (0 and 30 min) following DC \(^{126} \). Two of these three studies also observed an effect of DC on insulin, with one reporting a greater insulin AUC following DC \(^{126} \) and the other observing that DC raised insulin more than control immediately after meal ingestion \(^{8} \).

As discussed in Chapter 3, methodologies varied considerably across studies with no apparent methodological explanation for the different results. All three studies that found an effect of DC on glucose and/or insulin used filter coffee at relatively high strength and volume, all took venous samples and all studied healthy males \((n = 10 - 11)\), so these parameters do not explain the conflicting results. Of the two that found
DC to raise glucose, one gave their DC concurrently with the meal \(^8\) and the other gave it 60 min beforehand. A mix of habitual coffee drinkers and coffee-naïve participants were used. It is unlikely that a difference in caffeine content between DCs can explain the observed results, as both the study which found a decrease in glucose IAUC and the one that found an increase in IAUC measured plasma caffeine concentration throughout their tests and reported no difference at any time point between their DC and control legs.

The increase in glucose observed in two studies was unexpected, as the impairment in postprandial glucose metabolism observed following CC is generally attributed to caffeine. It is possible that there are other unidentified coffee components that may also have a glucose raising effect, however it is perhaps more likely that these are chance results, given that most studies have found no acute effect of DC. Indeed, in the current study, when post hoc comparisons were made for each combination of control v DC for the morning meal (4 combinations of treatment: 1v2 / 1v3 / 4v2 / 4v3), a significant difference between treatments was observed for glucose IAUC over both 120 min (paired t test, \(p=0.011\)) and 200 min (paired t test, \(p=0.039\)) for one of the four combinations. In the comparison of treatment 4 with 3, DC produced a higher glucose IAUC than control. Given that three of four comparisons produced no significant differences between control and DC, it seems likely, in this case, that this difference is due to daily variation in glycaemic response.

The reduced glucose IAUC following DC, reported by Battram et al. may be a result of a high CGA content in their coffee, however this cannot be verified as the concentration was not reported. Interestingly, three of their participants (total \(n=11\)) were tested at lunchtime having had a light breakfast. They did not report whether these participants reacted differently to the others, but this may have been a confounding factor to their results given the previously suggested potential for lunchtime coffee to have a beneficial effect on reduction of T2DM risk.

Most of the previous studies that found no effect of DC used instant coffee, with doses ranging from 2 – 12 g. As the dose used in the current study was small, 2 g, with a low amount of total CGA (47 mg) and caffeine (3.2 mg), it is perhaps unsurprising there was no observable effect of a single acute dose at breakfast-time.
5.5.3 Effect of decaffeinated coffee at lunchtime

There was no significant difference in the postprandial response to the second meal between treatments 1 (control/control) and 4 (control/DC) for either glucose or insulin. There was however a difference in steady-state beta cell function, with treatment 4 resulting in higher HOMA2_%B immediately prior to the lunchtime meal. There was also a trend for an effect on the AUC\textsubscript{IG} ratio with DC at lunchtime resulting in a higher AUC\textsubscript{IG} ratio than control.

*Post hoc* analysis of the morning data revealed a difference in insulin between treatments at 200 min (Wilcoxon Signed Rank, p=0.028), with treatment 4 displaying higher insulin than treatment 1. A difference was also observed for the glucose data with treatment 4 producing a smaller IAUC over the first 120 min (paired *t* test, p=0.028).

The difference in insulin observed at 200 min explains the difference in HOMA2_%B. However, as both treatments involved consumption of the control drink at breakfast, and the 200 min sample was taken immediately prior to the second test drink and meal, this difference must be attributed to normal daily variation in insulin, rather than an effect of DC. The trend for a difference in AUC\textsubscript{IG} ratio may also be partially explained by the difference in 200 min insulin between the two treatments.

There were no differences between treatments for the second meal postprandial glucose or insulin response, however *post hoc* analysis revealed a trend (p=0.060) for lower glucose at 245 min following DC (mean 6.95 mmol/l, SD 0.54) compared with control (mean 7.47 mmol/L, SD 0.74). This lower second meal peak following DC may be explained by normal daily variation in glucose response as the morning glucose IAUC was also lower for this treatment, however there was no difference in peak values in the morning.

In summary, a slight reduction in peak postprandial glucose was observed following DC at lunchtime, which may be indicative of some beneficial effect of DC. This would support the epidemiology suggesting a protective effect of coffee at lunchtime on T2DM risk \(^{(91)}\). However, the observed effect was small and may simply be a result of normal daily variation in glucose and insulin. To our knowledge, this has not been tested before, so further research is required before any conclusions can be drawn.
5.5.4 Effects of morning decaffeinated coffee on the postprandial response to a subsequent meal

There was no effect of DC consumed with a morning meal on the postprandial glucose and insulin responses to a subsequent meal. There was no difference in any measure over the entire study day (0-335 min), between treatments 1 (control/control) and 2 (DC/control), nor were there any differences in any measure when the morning and afternoon meals were examined separately.

This is in contrast to Moisey et al. who, whilst they did not find an overall carry-over effect of DC on a second meal, did observe a trend for higher glucose IAUC (p=0.07) and a significantly higher glucose peak at 215 min (35 min after the start of their second meal/OGTT) for DC than for control (8). They also reported higher glucose IAUC after their morning meal for DC. There were several differences between the study protocols which might explain this discrepancy. Moisey and colleagues collected venous blood samples and recruited males, whereas the current study recruited females and collected capillary blood samples. The participants were however of a similar age and healthy BMI range in the two studies. Perhaps the most important difference between protocols was in the type and dose of coffee. Moisey et al. used a single large dose of filter coffee whereas a more typical single serving of instant coffee was given in this study. However, as discussed in Section 5.5.2, where the effects of DC at a morning meal were examined, these differences in study design are unlikely to explain the difference in results as another study with a very similar design to theirs reported a contrasting reduction in postprandial glycaemic response following a similar dose of DC (10).

Additionally, in contrast to the current study, Moisey et al.’s participants’ glucose levels had returned to baseline values by 90 min following their first meal and appeared to exhibit a degree of reactive hypoglycaemia with values continuing to fall until 150 min and remaining below baseline at 180 min when they ate their second meal. Whilst the composition of their first meal (a high GI rice- and corn-based cereal with skimmed milk, providing 75 g available CHO) was comparable to that consumed in this study, the higher fat content of the current study meal may have slowed glucose absorption thus reducing the likelihood of reactive hypoglycaemia in this study.

Moisey et al. calculated their second meal IAUC using their fasted (-13 min) value as a baseline, in contrast to the 200 min values used in the current study. This gave them an overall treatment p-value of <0.001 (one-way RM ANOVA) when comparing CC, DC and control, with post hoc analysis revealing a trend for a greater postprandial
response in DC than control (p=0.07). When they re-ran their analysis using their 180 min values as baseline their overall p-value was reduced to a trend (p=0.07). They did not report results of any *post hoc* analysis, so it is unknown whether any difference between DC and control remained under this scenario. It is likely, however, that their trend for an overall effect of DC disappeared with this analysis. In contrast, the results of the current study did not change when the analysis was re-run using the fasted glucose values.

Although Moisey *et al.* did not report either the CGA or caffeine content of their DC, it is likely that both will be higher than that consumed in the current study, due to the relatively large volumes of DC (535 - 812 ml). However, as discussed in Section 5.5.2, a higher caffeine dose is unlikely to explain their results as they reported no difference in plasma caffeine levels between DC and control at any time-point.

Interestingly, examination of their data reveals an apparent reduction in the glucose peaks, for both DC and control, following the second meal in comparison with the first meal, although this was not tested statistically or discussed in their paper. This is somewhat anomalous with the increase in postprandial glycaemia that is normally observed throughout the day. Low GI and high RS breakfasts have been demonstrated to improve the postprandial glycaemic response to a standardised lunch in comparison to a high GI breakfast *(207,208)*, but this is a relative improvement only, with an increase in postprandial glycaemic response between breakfast and lunch still being observed. As Moisey *et al.* reported their breakfast to be high GI, this apparent reduction in postprandial response at lunchtime is unusual, particularly as their second meal was a 75 g dextrose OGTT, which one would expect to result in a higher glucose excursion than a mixed meal.

In summary, unlike Moisey *et al.*, the current study found that DC consumed with a morning meal did not result in an increased postprandial glucose response to a second meal compared to control. To our knowledge, no other studies have investigated this. It should be noted however that Moisey *et al.* also observed an increased postprandial response with DC for their first meal. This is in contrast to the majority of studies, which have reported no effect of DC at a single meal. Additionally, the apparent reduction in postprandial glucose excursion at their second meal also contradicts the literature. Clearly, further research is required before drawing conclusions as to the presence or absence of a second meal effect of DC.
5.5.5 Effect of multiple doses of decaffeinated coffee

There was no difference between either IAUC or time-point data over the entire study day (0-335 min), between treatments 1 (control/control), and 3 (DC/DC) for glucose or insulin. There was however a trend for an effect on the insulin time-point data and a trend for an effect on the AUC/I/G ratio following the second meal, with DC at breakfast and lunch resulting in lower insulin secretion after the second meal. This may be indicative of a metabolic effect of multiple dosing.

It may also indicate an effect of DC at lunchtime as was discussed in Section 5.5.3. However, if this is an effect of DC at lunchtime, it has manifested differently. When DC was consumed at lunchtime only (treatment 4), a reduction in the glucose peak response and an increase in the AUC/I/G ratio was observed, whereas two doses of DC has resulted in no effect on glycaemic response and a reduction in the AUC/I/G ratio. This may reflect actions of different coffee components, as the colonic metabolites from the first coffee dose would have been absorbed into the circulation by the time the second meal took effect, however, this is perhaps unlikely as there was no observed reduction in insulin at the second meal for treatment 2, which also gave DC at the first meal.

There was no difference in either IAUC or time-point data over the entire study day (0 - 335 min), between treatments 1 (control/control), 2 (DC/control) and 3 (DC/DC) for glucose or insulin. Nor were there any differences in any insulin sensitivity or beta cell function measures, indicating no dose-response effect of DC when individual doses are taken with two meals. This is in line with the first study, reported in Chapter 4, which found no dose-response effect of increasing amounts of coffee at a single meal. As discussed in Chapter 4, it is likely, with 10 participants, that this study was also underpowered to find a dose-response effect.

5.6 Study limitations

As discussed previously, the DC was relatively low in total CGA content which may explain the lack of significant effect. The only study in the systematic review to analyse their coffee for CGA content found no effect of DC on glucose and insulin response \(^{(147)}\); their DC provided 353 mg total CQA in a 12 g serving, making it perhaps unlikely that the current study would demonstrate an effect with its much lower CQA content (47 mg).
For practical reasons, the effects of two servings given more than three hours apart were examined. It is possible that more of an effect would be observed from more frequent servings across a longer study day, particularly as the glycaemic response deteriorates later in the day.

Individuals vary in their glycaemic response from day to day, so many studies that measure GI test subjects on more than one occasion to minimise this effect (209). If each test had been repeated, one could be more confident that the observed effects were a genuine effect of the treatment. However as previously noted, this study was designed as an undergraduate final year project and thus had certain time constraints.

5.7 Conclusion

In contrast to the first study, where an increase was observed in the postprandial glycaemic response following a single serving of CC, the current study found no effect of a single, “normal” serving of instant DC on postprandial glucose and insulin responses to a mixed meal in young healthy women. This supports the hypothesis that a “normal” serving of DC would have no effect on the postprandial glycaemic response. It is also in agreement with the majority of previous studies which have reported no acute effects of a single larger serving of DC. There was also no carry-over effect of morning DC, in contrast to Moisey et al., who reported an increased postprandial response to a second meal following morning DC. However, as previously discussed, Moisey et al. were also one of the few groups to find an effect of morning DC. With no other studies for comparison, it is not possible at present to determine whether a carry-over effect exists.

Some interesting trends were observed for an effect of DC at lunchtime, with a single lunchtime serving resulting in a reduction in postprandial glycaemic peak and two servings of DC, taken at breakfast and lunch, resulting in a reduced postprandial insulin response to the lunchtime meal. To our knowledge, this is the first study to investigate the effects of lunchtime DC and the effects of repeated dosing, so it is quite possible that the findings are not reflective of a true effect of DC and are instead a result of random daily variation in response. It should also be noted that the magnitude of the observed effect was small, so it is perhaps questionable whether this effect is physiologically relevant. Nevertheless, if verified by future studies, these beneficial effects of lunchtime DC may partly explain the reduction in T2DM risk associated with lunchtime coffee drinking.
Whilst it appears that a single acute dose of DC in the morning has little effect on the postprandial glycaemic and insulinaemic responses, it is not clear whether it has any effects when taken later in the day. Further investigations examining the effects of more frequent dosing, continuing later in the day, are recommended. This would more closely reflect normal coffee consumption patterns and would provide a more accurate picture of the acute effects of coffee drinking throughout the day.
Chapter 6. The effects of 12 weeks of coffee drinking on glucose and lipid metabolism in coffee-naïve individuals: primary analysis

6.1 Introduction

There have been few longer-term intervention studies into the effects of coffee on glucose metabolism. The majority of these have examined the effects in habitual coffee drinkers (11–13) and have examined the effects on fasted glucose and insulin levels, not the postprandial response. It could be argued that habitual coffee drinkers will already have gained any potential longer-term benefits of coffee drinking and may therefore not be the most appropriate population to study; however, despite this, there have been very few interventions in coffee-naïve individuals.

One study, which did examine the effects of coffee in non-coffee drinkers, observed a reduction in the postprandial glycaemic response to an OGTT, following a 16-week intervention where participants drank five cups of coffee per day (112). This reduction was observed for both CC and DC.

It should also be considered that any potential beneficial effects of coffee, in terms of reducing risk of developing T2DM, may not be directly related to glucose metabolism, but may be through actions on other risk factors related to development of T2DM, such as features of the MS. Metabolic Syndrome is characterised by a combination of disorders which increase the risk of developing T2DM. These include raised fasted glucose, raised TAG, low HDL cholesterol and raised BP.

Gender differences have been reported in glucose and lipid metabolism; men having been observed to have higher fasted glucose, higher postprandial insulin and TAG, less postprandial NEFA suppression and lower fasted TAG and HDL cholesterol than women (210–212). There is also potential for coffee to affect men and women differently as several epidemiological studies have reported differences between the genders with respect to coffee drinking and T2DM risk (213,214). To date, however, no longer-term intervention has examined whether coffee drinking differentially affects men and women with respect to glucose metabolism.
6.2 Aims

The primary aim of this study was to investigate the effects of 12 weeks of CC consumption on glucose metabolism and features associated with the MS in coffee-naive individuals.

A secondary aim was to investigate whether men and women reacted differently to the intervention.

A further secondary aim (covered in Chapter 7) was to examine whether there were any differences between slow and fast caffeine metabolisers, both at baseline and in their response to the intervention.

6.3 Methods

6.3.1 Participants

Participants were recruited from the student and staff population at the University of Surrey and from the local community, by word of mouth, poster advertisement and internal emails. Written informed consent was obtained from all participants. The study was given a favourable ethical opinion by the University of Surrey Ethics Committee (EC/2013/68/FHMS) and registered with the ISRCTN, trial number ISRCTN42321643 (http://www.controlled-trials.com/). Recruitment and data collection were carried out between September 2013 and July 2014.

Participants were required to be healthy adult males and females, aged over 18 y, taking no prescription medication other than oral contraceptives. They had to have been weight stable for the previous 3 months and to have no history of heart disease, diabetes, liver disease or any gastrointestinal or endocrine disorders. They were ineligible if they were regular smokers or if they drank more than 4 cups per week of either tea or coffee.

6.3.2 Study design

A 12 week open-label parallel-arm intervention investigating the effects on non-coffee drinkers of drinking 4 cups of caffeinated coffee per day on various risk factors for T2DM.
6.3.3 General protocol

The timeline for the study is shown in Figure 6.3.3-1.

![Figure 6.3.3-1 Study timeline for 12 week coffee intervention.](image)

Potential participants were invited to attend a screening session (detailed in Chapter 2) where the study was explained to them in detail. If they fulfilled the inclusion criteria and decided to take part in the study, a date was set for their first study day. They were given some instant CC portions to take home and instructed to commence a two day CC trial (Section 2.3.3.1), 16 days before their first study date. They were contacted by telephone after they had completed the two day trial to find out whether they had suffered any adverse effects of the CC and to confirm they still wanted to take part in the study. If they wished to continue they were then fully enrolled onto the study. They then undertook a 14 day caffeine-washout period, where they were instructed to abstain from all caffeine-containing foods and drinks, such as tea, coffee, energy drinks and chocolate. They were advised not to drink decaffeinated teas and coffees as these also contain small amounts of caffeine.

Participants were randomised to either the CC or control group prior to their first study day using a stratified random block design to ensure sufficient numbers in each group and an even split of males and females. Thirty participants were recruited in total, with 20 allocated to the CC intervention and 10 to the control group. One from the CC group dropped out immediately prior to their second study day for personal reasons, resulting in 29 participants completing the trial.

After the 14 day washout period each participant attended their first study day. Details of the study day protocol are provided in Section 6.3.3.3. At the end of the study day, they were notified of their allocated group and given instructions for the following 12 weeks. The CC group were given a supply of individual CC portions and asked to consume four per day, for the next 12 weeks and to continue to refrain from all caffeine-containing food and drinks, apart from the supplied CC, for the duration of the study; the control group were instructed to remain caffeine-free during this period.
During the trial, all participants were randomly visited either once or twice, with little advance notice, and asked to provide a saliva sample which was later analysed for caffeine content in order to monitor compliance.

At the end of the trial, participants in the CC group were instructed to stop drinking the supplied CC two days before their final study day to ensure they were completely caffeine-free for the study day. The procedure for the second study day was identical to the first, except that no buccal swabs were taken. The coffee group were instructed to return all unused CC portions. Participants were given £150 on completion of the trial, by way of thanks for their participation.

### 6.3.3.1 Two day trial

Before starting the intervention, all participants underwent a two day trial to check for any adverse effects of the CC and to ensure they were able to comply with the requirements of the intervention. They were given eight individual portions of instant CC and instructed to consume four per day over two days. They were instructed to dissolve the CC granules in a cup of hot water and to drink the CC without addition of milk or sugar. They were free to choose the timing of their drinks, but were advised to spread consumption throughout the day and to try to consume them all before 4 pm to avoid any potential sleep disturbance from the caffeine. They were followed up after the trial to check whether they had suffered any adverse effects and to confirm whether they still wished to take part in the study.

### 6.3.3.2 Coffee portions

The coffee portions used in this study were a commercially available instant CC (Carte Noire, Jacobs Douwe Egberts GB Ltd, UK). Each portion contained 2 g instant CC granules, the equivalent of a teaspoon. The coffee was analysed by Sciantec Analytical Services (Selby, UK) for caffeine, trigonelline and CGA content (detailed in Table 6.3.3.2-1) and for total and free CHO content (see Appendix 4).
6.3.3.3 Study day protocol

For two days prior to each study day, the participants were asked to refrain from exercise and all caffeine-containing food and drinks. On the day prior to each study day they were also asked to avoid alcohol. They were provided with a standardised meal to consume on the evening prior to each study day, which comprised of macaroni cheese (Tesco, Italian range, 450 g) providing 784 kcal, 31.2 g protein, 35.2 g fat, 80.5 g CHO and 10.1 g fibre.

After a 12 h overnight fast, participants arrived at the SCRC between 8:00 and 9:00 am. After a ten minute rest, BP, weight and body composition were measured and a buccal swab was taken for later DNA analysis (first visit only) according to the methods set out in Chapter 2. A cannula was inserted into an antecubital vein by a trained phlebotomist and an initial fasted blood sample was taken. Participants then consumed the liquid meal within 10 minutes. Further venous blood samples were taken at 15, 30, 60, 90, and 120 minutes after the first mouthful of the liquid meal. Each study session lasted approximately 3 h, during which time the participants were required to stay in the SCRC. They were permitted to bring work/books/laptops with them but were required to minimise physical activity.

6.3.3.4 Liquid meal

The liquid meal consisted of 2 x 200 ml bottles of Fortisip (Nutricia, Trowbridge, UK). Participants were allowed to choose from three flavours, toffee, vanilla and banana on the first study day, which they then had to repeat at the second study day. The macronutrient composition of the meal is reported in Table 6.3.3.4-1.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Amount per portion (mg)</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caffeine</td>
<td>43.8</td>
<td>2.19</td>
</tr>
<tr>
<td>Trigonelline</td>
<td>14.5</td>
<td>0.73</td>
</tr>
<tr>
<td>3-CQA</td>
<td>10.9</td>
<td>0.55</td>
</tr>
<tr>
<td>4-CQA</td>
<td>13.5</td>
<td>0.68</td>
</tr>
<tr>
<td>5-CQA</td>
<td>16.9</td>
<td>0.85</td>
</tr>
<tr>
<td>3,4-diCQA</td>
<td>0.8</td>
<td>0.04</td>
</tr>
<tr>
<td>3,5-diCQA</td>
<td>1.0</td>
<td>0.05</td>
</tr>
<tr>
<td>4,5-diCQA</td>
<td>0.6</td>
<td>0.03</td>
</tr>
<tr>
<td>Chlorogenic Acid (Total)</td>
<td>43.6</td>
<td>2.18</td>
</tr>
</tbody>
</table>

CQA: caffeoylquinic acid

Table 6.3.3.2-1  Instant coffee analysis.
All blood samples were taken by an experienced phlebotomist. Serum samples were collected into Z Serum Separator Clot Activator Vacuette tubes and plasma samples into heparin fluoride tubes. Serum samples were left to stand at room temperature for 30 min to allow clotting before being transferred onto ice. Plasma samples were put straight onto ice. At the end of the study day all samples were centrifuged and aliquoted into storage tubes before being frozen at -20 °C until they were batch analysed at the end of the study.

**Statistical analysis**

All statistical analysis was carried out using SPSS Statistics software, version 22. The Shapiro-Wilks test was used to check normality of data. One- and two-way RM ANOVA was used to compare groups over all time-points. Individual time-point data were also analysed to determine the source of statistical differences for purposes of hypothesis generation. *T* tests were used for comparisons between groups when the data were normally distributed and the equivalent non-parametric tests were used when they were not. Fisher’s Exact tests were used for assessment of independence of variables. Primary analysis compared the CC and control groups. Additional analysis included gender as a cofactor to investigate whether men and women reacted differently to the intervention, given the known gender differences in glycaemic and lipidaemic response. Statistical significance was classed as a p-value < 0.05.

**Table 6.3.3.4-1 Liquid test meal macronutrient composition.**

<table>
<thead>
<tr>
<th>Macronutrient</th>
<th>Amount in meal</th>
<th>Macronutrient as % of total energy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
<td>600</td>
<td></td>
</tr>
<tr>
<td>Protein (g)</td>
<td>24</td>
<td>16</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>23</td>
<td>35</td>
</tr>
<tr>
<td>Saturated fat (g)</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>74</td>
<td>49</td>
</tr>
<tr>
<td>Sugars (g)</td>
<td>27</td>
<td>18</td>
</tr>
</tbody>
</table>
6.4 Results

6.4.1 Participant compliance

Salivary caffeine

Participant compliance was assessed by measurement of caffeine in random saliva samples. All participants provided at least one sample, with 79% of the CC group and 70% of the controls providing two. Within the CC group, all participants had at least one sample with a caffeine concentration over 3 µM; three had one sample less than 1 µM. In the control group, all samples were less than 1 µM. The mean salivary caffeine concentrations for each participant are shown in Figure 6.4.1-1; panel A displays the concentrations for all participants, whereas panel B displays the participants with concentrations less than 10 µM, allowing the spread of concentrations at this lower level to be displayed more clearly.

![Figure 6.4.1-1 Mean salivary caffeine concentrations. Each point represents the mean caffeine concentration for one participant. Panel A: caffeine concentrations for all participants (n=27); panel B: participants with caffeine concentrations < 10 uM.](image)

Coffee portions

In the CC group, the number of returned CC portions was used to determine daily intake. Median daily intake was 3.9 servings (97.5% of target), with individual participant mean daily intakes ranging from 3.5 – 4.3 servings/day.
6.4.2 Genotyping

Participant DNA, extracted from buccal swabs taken at the first study day, was genotyped for the rs762551 SNP in the CYP1A2 gene (method detailed in Chapter 2). The allelic discrimination plot for the assay is shown in Figure 6.4.2-1.

![Figure 6.4.2-1 Allelic discrimination plot for the rs762551 SNP of the CYP1A2 gene. The plotted points represent the genotypes allocated to each of the study participants (n=27).](image)

The distribution of genotypes was as expected within the population and did not deviate from the Hardy-Weinberg equilibrium according to a Pearson $\chi^2$ test with 1 df for either the Asian ($p=0.439$) or Caucasian ($p=0.592$) participants. There were two participants with the C/C genotype, both of which had been randomised to the control group. Although one C allele is deemed sufficient to be considered a slow caffeine metaboliser \[^{155}\], it has been suggested that this A/C genotype confers an intermediate phenotype to A/A (fast) and C/C (slow); this has been demonstrated when phenotyping is based on caffeine clearance ratios, where a trimodal distribution has been observed \[^{215}\]. It was therefore decided to exclude the two C/C participants from all analyses because both were in the control group, resulting in an imbalance between coffee and control groups for this genotype, which could bias the data when comparing groups. The removal of these two participants from the control group resulted in a more equal balance of genotypes between groups. The A/C genotype will be considered to confer a slow phenotype for the remainder of this thesis.
Most of the CC group (52.6%) were fast metabolisers, whereas only 37.5% of the control group were, however this difference was not statistically significant (Fisher’s Exact, p=0.678). The majority of females were slow metabolisers (69.2%), whereas most males were fast metabolisers (64.3%), but this difference in the proportion of fast to slow metabolisers between males and females again was not statistically significant (Fisher’s Exact, p=0.128). The relative proportion of Caucasian to Asian participants was not statistically different between fast and slow metabolisers (Fisher’s Exact, p=1.000); nor were there any significant differences in ethnic distribution between CC and control groups (Fisher’s Exact, p=1.000). The distribution of genotypes and ethnicities across treatment group is reported in Table 6.4.2-1.

<table>
<thead>
<tr>
<th>Allocation</th>
<th>Gender</th>
<th>Number of participants</th>
<th>A/A (fast)</th>
<th>A/C (slow)</th>
<th>Caucasian</th>
<th>Asian</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coffee</td>
<td>Male</td>
<td>10</td>
<td>7</td>
<td>3</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>9</td>
<td>3</td>
<td>6</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td><strong>Coffee group total</strong></td>
<td><strong>19</strong></td>
<td><strong>10</strong></td>
<td><strong>9</strong></td>
<td><strong>11</strong></td>
<td><strong>8</strong></td>
</tr>
<tr>
<td>Control</td>
<td>Male</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td><strong>Control group total</strong></td>
<td><strong>8</strong></td>
<td><strong>3</strong></td>
<td><strong>5</strong></td>
<td><strong>5</strong></td>
<td><strong>3</strong></td>
</tr>
</tbody>
</table>

### 6.4.3 Participant characteristics

Baseline values for age, height, weight, SBP, DBP, BMI and body fat percentage were examined. All anthropometric data were normally distributed apart from age. There were no statistically significant differences between CC and control groups at baseline (p>0.388). When genders were compared there were differences between males and females, with males having a lower body fat percentage (p=0.002) and higher fasted glucose (p=0.047) and SBP (p=0.030) than females. There were no significant differences between control and CC groups for any of the tested parameters for either females (p>0.150) or males (p>0.062). The baseline participant characteristics are reported in Table 6.4.3-1.
Pre-intervention coffee and caffeine intake

Pre-intervention coffee and caffeine intake were estimated as described in Section 2.2.2; neither were normally distributed. Median caffeine intake was 80 mg per week (range 0 – 410 mg) and median coffee intake was zero servings per week (range 0 – 4 servings) for all participants. There were no significant differences between CC and control groups for either caffeine or coffee intake (Mann-Whitney U, p>0.549).

6.4.4 Comparison of coffee and control groups

6.4.4.1 Anthropometrics

Weight, body fat, BMI, SBP and DBP were measured at each of the two study visits. There were no within-group (paired t test, p>0.267) or between-group (RM ANOVA, p>0.216) differences. Changes between the two visits are reported in Table 6.4.4.1-1. There was high inter-individual variation in these measures.

Table 6.4.4.1-1 Changes in anthropometric measures between the two study visits by treatment group.

<table>
<thead>
<tr>
<th></th>
<th>Coffee group (n=19)</th>
<th>Control group (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean change</td>
<td>SD</td>
</tr>
<tr>
<td>Age at start (y)</td>
<td>0.1</td>
<td>2.0</td>
</tr>
<tr>
<td>Height (m)</td>
<td>0.1</td>
<td>0.7</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>0.0</td>
<td>1.8</td>
</tr>
<tr>
<td>BMI (kgm⁻²)</td>
<td>-0.1</td>
<td>8.5</td>
</tr>
<tr>
<td>Body fat %</td>
<td>-0.6</td>
<td>8.2</td>
</tr>
</tbody>
</table>

BMI: body mass index; BP: blood pressure.
6.4.4.2 Glucose

All glucose time-point data were normally distributed apart from the 15 min sample on visit 2; log transformation of the time-points did not result in all data being normally distributed. As 11/12 time-points were normally distributed, parametric tests were carried out on the original dataset.

Baseline data

There were no differences between coffee and control groups at visit 1, either for fasted glucose (unpaired t test, p=0.792) or IAUC for the 2 h postprandial response (unpaired t test, p=0.906) nor was there any treatment * time effect (p=0.830) when the time-points were analysed by RM ANOVA. The 2 h postprandial glucose response for each group is shown in Figure 6.4.4.2-1.

Figure 6.4.4.2-1 Baseline postprandial glucose response by treatment group.
There were no significant differences between treatments either for fasted glucose or IAUC (unpaired t test, p>0.791). Error bars are SEM (coffee group: n=19; control group: n=8).
When CC and control groups were combined, males had a higher fasted glucose (mean 4.75 mmol/L, SD 0.28) than females (mean 4.45 mmol/L, SD 0.46) at baseline (unpaired t test, p=0.047). There was no difference in baseline IAUC between genders, however there was a trend for an effect of gender when time-points were analysed (RM ANOVA, p=0.079). The 2 h glucose response for each gender is shown in Figure 6.4.4.2-2.

Figure 6.4.4.2-2 Baseline postprandial glucose response by gender. There was a significant difference between genders for fasted glucose (t test, p=0.047) and a trend for a difference between genders over all time-points (RM ANOVA, p=0.079). Error bars are SEM (males: n=14; females: n=13).
**Effect of treatment**

Fasted glucose values were compared between groups and visits by one-way RM ANOVA. A within-group effect of visit was found (p=0.010), with both CC and control groups displaying an increase from visit 1 to 2, but there was no between-group difference (visit * treatment p=0.435). Adding gender as a cofactor did not change this result. Fasted glucose by visit and group is shown in **Figure 6.4.4.2-3**.

![Figure 6.4.4.2-3](image)

**Figure 6.4.4.2-3  Fasted glucose by visit and treatment group.**

There was a difference between visits for all participants (RM ANOVA, p=0.010), but no difference between groups. Error bars are SEM (coffee group: n=19; control group: n=8).
There was no difference between groups and visits in the postprandial period when the time-point data were analysed (one-way RM ANOVA, $p>0.333$). However, when the IAUCs were analysed, a within group effect was again found (one-way RM ANOVA, $p=0.019$), with both CC and control groups displaying a decrease from visit 1 to 2, but again there was no between-groups difference (visit * treatment, $p=0.694$), nor was there any effect of gender when it was added as cofactor. The 2 h glucose response for each visit, by group, is shown in **Figure 6.4.4.2-4**.

![Postprandial glucose response by visit and treatment group.](image)

**Figure 6.4.4.2-4** Postprandial glucose response by visit and treatment group.
Panel A: coffee; panel B: control. There was a difference in IAUC between visits for all participants (RM ANOVA, $p=0.019$), but no between groups difference. Error bars are SEM (coffee group: $n=19$; control group: $n=8$).
6.4.4.3 Insulin

Half (8/16) of the insulin time-point data were normally distributed; log transformation of the time-points resulted in all data being normally distributed. Parametric tests were therefore carried out on the log-transformed data.

**Baseline data**

There were no differences between CC and control groups at visit 1, either for fasted insulin (unpaired t test, p=0.307) or IAUC for the 2 h postprandial response (unpaired t test, p=0.293) nor was there any treatment * time effect (p=0.830) when the time-points were analysed by one-way RM ANOVA. There were no differences between genders for any of the baseline measures (data not shown). The 2 h postprandial insulin response for each group is shown in Figure 6.4.4.3-1.

![Figure 6.4.4.3-1 Baseline postprandial insulin response by treatment group.](image)

There were no significant differences between treatments either for fasted insulin or IAUC (p>0.292). Error bars are SEM (coffee group: n=19; control group: n=8).
**Effect of treatment on serum insulin**

Fasted insulin values were compared between visits and groups by one-way RM ANOVA. An effect of visit was found (p=0.011), in addition to a visit * treatment effect (p=0.015). *Post hoc* analysis revealed a trend for an increase in the control group after adjustment for multiple comparisons (paired *t* test, p=0.070), with no significant difference between visits for the CC group. Adding gender as cofactor did not affect the result. The change between visits is shown in **Figure 6.4.4.3-2**.
There was no difference in IAUC between groups over the two visits, but there was a strong trend for a visit * time * treatment effect (RM ANOVA, p=0.054) with the CC group displaying a later peak at visit 2 (60 min) than at visit 1 (30 min). Adding gender as cofactor did not affect this result. The postprandial insulin responses by visit for each group are shown in Figure 6.4.4.3-3.

Figure 6.4.4.3-3 Postprandial insulin response by visit and treatment group.
Panel A: coffee group. Panel B: control group. There was no difference in IAUC between groups, but there was a trend for a difference in the response over time between the groups when time-points were analysed (RM ANOVA, visit * time * treatment interaction, p=0.054). Error bars are SEM (coffee group: n=19; control group: n=8).
6.4.4.4 Measures of insulin sensitivity and response

Measures of insulin sensitivity and beta cell function were calculated as described in Chapter 2. These measures were not normally distributed at one or both visits, but all were normally distributed after log transformation so parametric tests were applied to the log-transformed data.

Baseline data

There were no differences between the CC and control groups at baseline for any measure (unpaired t test, \( p>0.090 \)), nor were there any differences between genders.

Effect of treatment

Repeated measures ANOVA was used to compare the difference in measures between visits and groups. There was no difference in any measure apart from HOMA2-%S (fasted insulin sensitivity) which had an overall effect of visit (\( p=0.006 \)) and a visit by treatment effect (\( p=0.015 \)). Post hoc analysis revealed a significant decrease in insulin sensitivity in the control group (paired t test, \( p=0.031 \)), with no change in the CC group (\( p=0.678 \)), however the decrease in the control group was no longer significant after adjustment for multiple comparisons. These results were not changed when gender was added as cofactor. Mean values by visit for each group are reported in Table 6.4.4.4-1.

Table 6.4.4.4-1  Insulin sensitivity measures by visit and treatment group.

<table>
<thead>
<tr>
<th></th>
<th>Coffee (n=19)</th>
<th>Control (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Visit 1 Mean</td>
<td>Visit 2 Mean</td>
</tr>
<tr>
<td>HOMA-2 %S</td>
<td>82.25</td>
<td>80.56</td>
</tr>
<tr>
<td>HOMA-2 %B</td>
<td>141.87</td>
<td>130.44</td>
</tr>
<tr>
<td>Matsuda</td>
<td>5.70</td>
<td>5.52</td>
</tr>
<tr>
<td>AUCI/G</td>
<td>79.94</td>
<td>35.17</td>
</tr>
<tr>
<td>Matsuda*AUCI/G</td>
<td>393.36</td>
<td>383.46</td>
</tr>
</tbody>
</table>

* Significant difference between visits by group (RM ANOVA, \( p<0.05 \)). HOMA: Homeostatic model of assessment; %S: sensitivity; %B: beta cell function; AUCI/G: ratio of insulin to glucose area under curve; Matsuda * AUCI/G: product of Matsuda index and AUCI/G ratio.
None of the TAG time-point or IAUC data were normally distributed. Log transformation resulted in all data, apart from IAUC at visit 2, being normally distributed, therefore parametric tests were carried out on the log-transformed time-point and baseline IAUC data. Non parametric tests were carried out on other IAUC data.

**Baseline data**

There were no differences between CC and control groups at visit 1 for fasted TAG (unpaired t test, p=0.803). There was however a difference in IAUC between the groups for the 2 h postprandial response (unpaired t test, p=0.008) with the CC group having a higher mean IAUC (26.4 mmol/L.120 min) than the control group (8.7 mmol/L.120 min). There was also a trend for a group * time effect (p=0.085) when the time-points were analysed by RM ANOVA, with the TAG appearing to rise faster in the CC group than in the control. The 2 h postprandial TAG response for each group is shown in Figure 6.4.4.5-1.
When groups were combined and genders compared, there was no difference between men and women for fasted TAG (unpaired $t$ test, $p=0.320$), however there was a trend for lower IAUC in women (unpaired $t$ test, $p=0.084$) and a trend for a time * gender effect when time-point data were analysed (one-way RM ANOVA, $p=0.061$), with TAG appearing to rise faster in men. The 2 h postprandial TAG response split by gender is shown in Figure 6.4.4.5-2.

![Figure 6.4.4.5-2 Baseline postprandial TAG response by gender. There was a trend for a time * gender effect (one-way RM ANOVA, $p=0.061$). Error bars are SEM (males: $n=14$; females: $n=13$).](image)

When baseline IAUC for TAG was analysed by two-way ANOVA with both group and gender as cofactors, the effect of group remained ($p=0.006$) and an effect of gender was also found ($p=0.028$), with females having a lower IAUC than males. Baseline IAUC for each group, split by gender is shown in Figure 6.4.4.5-3.
Chapter 6.

Effect of treatment

Fasted TAG values were compared between visits by one-way RM ANOVA. No effect of visit, treatment or visit * treatment effect was found (p>0.521). The mean fasted TAG values for each group are reported in Table 6.4.5.1.

![Figure 6.4.5-3 Baseline TAG IAUC by treatment group and gender.](image)

Two-way ANOVA revealed a difference between groups (p=0.006) and genders (p=0.028), with the coffee group displaying a greater IAUC than the control group and males producing a greater IAUC than females. Error bars are SEM (coffee group males: n=10; coffee group females: n=9; control group males: n=4; control group females: n=4).

Table 6.4.5-1 Mean fasted TAG by visit and treatment group.

<table>
<thead>
<tr>
<th>Group</th>
<th>Visit</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coffee (n=19)</td>
<td>1</td>
<td>0.98</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.08</td>
<td>0.48</td>
</tr>
<tr>
<td>Control (n=8)</td>
<td>1</td>
<td>0.95</td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.97</td>
<td>0.39</td>
</tr>
</tbody>
</table>

TAG: triacylglycerol; SD: standard deviation.
There was a significant difference between groups for IAUC at visit 2 (Mann-Whitney U, \( p=0.034 \)), as at visit 1, however there was no difference in the change from visit 1 to visit 2 between groups (Mann-Whitney U, \( p=0.449 \)). The IAUC by visit for each group is shown in Figure 6.4.4.5-4.

![Figure 6.4.4.5-4 TAG IAUC by visit and treatment group.](image)

There was a difference between groups at each visit (Mann-Whitney U, \( p<0.035 \)), but no significant difference between groups for change in IAUC between visits. Different letters denote a significant difference between groups for that visit. Error bars are SEM (coffee group: \( n=19 \); control group: \( n=8 \)).

There was no difference between groups and visit when the time-points were compared by one-way RM ANOVA, however there was a trend for a time * group effect (\( p=0.061 \)); examination of the time-point data for visit 2 (not shown) suggests the coffee group TAG to be rising faster than controls as at visit one. Adding gender as cofactor did not change the significance of the results.
6.4.4.6 Non-Esterified Fatty Acids (NEFA)

Only 2 of the 12 NEFA time-points and neither AUC were normally distributed, however baseline fasted NEFA was normally distributed. Log transformation (base 10 and natural log) did not improve the normality of the data. Non-parametric tests were therefore carried out wherever possible. When this was not possible, for example with repeated measures on time-point data, then parametric tests were carried out on the original data.

Baseline data

There were no differences between CC and control groups at visit 1 for fasted NEFA (unpaired t test, p=0.979) or total AUC (Mann-Whitney U, p=0.418), nor was there any difference between groups when time-course data were compared (RM ANOVA, p>0.742). The 2 h postprandial NEFA response for each group is shown in Figure 6.4.4.6-1.

![Figure 6.4.4.6-1](image)

*Figure 6.4.4.6-1* Baseline postprandial NEFA response by treatment group. There were no significant differences between groups for either fasted NEFA or 2 h AUC (p>0.417). Error bars are SEM (coffee group: n=19; control group: n=8).
When men and women were compared there was no difference for either fasted NEFA (unpaired \( t \) test, \( p=0.591 \)) or 2 h AUC (Mann-Whitney \( U \), \( p=0.220 \)). However, when time-points were analysed by RM ANOVA, a time * gender effect was found (\( p=0.009 \)) along with a slight trend for gender (\( p=0.098 \)). The baseline NEFA response for men and women is shown in Figure 6.4.4.6-2; it reveals greater postprandial NEFA suppression in females than in males.

**Figure 6.4.4.6-2** Baseline postprandial NEFA response by gender.

There was a time * gender effect (one-way RM ANOVA, \( p=0.009 \)). Error bars are SEM (males: \( n=14 \); females: \( n=13 \)).
**Effect of treatment**

There was no difference between groups at visit 2 for either fasted NEFA or 2 h AUC, nor was there any difference between groups in change from baseline for these measures (Mann-Whitney U, p>0.695). The AUC at each visit by group is shown in Figure 6.4.4.6-3.

![Figure 6.4.4.6-3 NEFA AUC by treatment group and visit.](image)

No significant differences were found between groups for either fasted or 2 h AUC (Mann-Whitney U, p>0.695). Error bars are SEM (coffee group: n=19; control group: n=8).

When time-points were compared between visits, no effect of visit, treatment or visit * treatment effect was found for any measure (one-way RM ANOVA, p>0.503), however when gender was added as a cofactor to treatment group, a time * gender effect was found (p=0.010). Examination of the time-point data for visit 2 (not shown) suggests that females were displaying greater NEFA suppression than males at visit two, similar to that observed at baseline.
6.4.4.7 Total cholesterol

Fasted cholesterol was normally distributed at both visits.

Baseline data

There were no differences between coffee and control groups at visit 1 for fasted cholesterol (unpaired t test, p=0.983), nor was there any difference between genders (unpaired t test, p=0.465).

Effect of treatment

Fasted total cholesterol was compared between visits and groups by one-way RM ANOVA. No effect of visit, treatment or visit * treatment effect was found (p>0.477), nor was there any difference between groups at visit 2 (unpaired t test, p=0.549). Re-performing the analysis with gender as cofactor did not change the significance of any results. Fasted total cholesterol for each group at each visit is shown in Figure 6.4.4.7-1.

![Figure 6.4.4.7-1](image)

Figure 6.4.4.7-1 Mean total cholesterol by visit and treatment group.

There was no difference between groups at baseline (unpaired t test, p=0.983) nor was there any effect of treatment (RM ANOVA, p>0.477). Error bars are SEM (coffee group: n=19; control group: n=8).
6.4.4.8 HDL cholesterol

HDL cholesterol was normally distributed at visit 2, but not at visit 1; both were normally distributed after log transformation therefore parametric tests were conducted.

**Baseline data**

There were no differences between CC and control groups at visit 1 for HDL cholesterol (unpaired $t$ test, $p=0.218$), nor was there any difference between males and females (unpaired $t$ test, $p=0.254$).

**Effect of treatment**

HDL cholesterol was compared between visits and groups by one-way RM ANOVA. No effect of visit, treatment or visit * treatment effect was found ($p>0.159$), nor was there any difference between groups at visit 2 (unpaired $t$ test, $p=0.137$). Re-performing the analysis with gender as cofactor did not change the significance of any results. HDL cholesterol for each group at each visit is shown in Figure 6.4.4.8-1.

![Figure 6.4.4.8-1 Mean HDL cholesterol by visit and treatment group.](image)

There was no difference between groups at baseline (unpaired $t$ test, $p=0.218$) nor any effect of treatment or visit (RM ANOVA, $p>0.159$). Error bars are SEM (coffee group: $n=19$; control group: $n=8$).
6.4.4.9 Inflammatory markers, IL-6 and TNF-α

Neither IL-6 nor TNF-α were normally distributed for either visit. Log transformation resulted in normal distribution of TNF-α but not IL-6. Non-parametric tests were therefore carried out on IL-6 data and parametric tests were carried out on the log-transformed TNF-α data.

**Baseline data**

There were no differences between coffee and control groups at visit 1 for IL-6 (Mann-Whitney U, p=0.775) or TNF-α (unpaired t test, p=0.499), nor were there any significant differences between genders. There was however a slight trend (unpaired t test) for women to have lower TNF-α (mean 1.47 pg/ml; SD 0.98) than men (mean 3.53 pg/ml; SD 3.64).

**Effect of treatment**

No effect of visit, treatment or visit * treatment was found for TNF-α (RM ANOVA, p>0.155) however there was a trend for a difference between treatments for TNF-α at visit 2 (unpaired t test, p=0.073). The addition of gender as a cofactor did not significantly change these results. There was no difference between groups for IL-6 either at visit 2, or for the change between visits (Mann-Whitney U, p>0.359). Mean IL-6 and TNF-α for each group at each visit is shown in Figure 6.4.4.9-1.
Figure 6.4.4.9-1 Mean IL-6 and TNF-α by visit and treatment group.
Panel A: IL-6; Panel B: TNF-α. There were no differences between treatment groups, either at baseline or in their response to the intervention, however there was a trend for a difference between groups at visit 2 for TNF-α (unpaired t test, $p=0.073$). Error bars are SEM (coffee group: $n=19$; control group: $n=8$).
6.5 Discussion

6.5.1 Summary of results

There was no difference between CC and control groups at baseline for any measure other than TAG, where the postprandial IAUC was higher in the CC group than in controls. When genders were compared at baseline there were several differences, with women exhibiting lower fasted glucose and greater postprandial NEFA suppression than men and a trend for lower postprandial glucose and TAG. Women also had higher body fat and lower SBP.

Fasted insulin increased and insulin sensitivity (HOMA2_%S) decreased in the control group, but not in the CC group. A delayed insulin peak was observed in the CC group at the second visit. Fasted glucose increased and IAUC glucose decreased in both groups between visits, but there was no difference between groups. There was no effect of CC on TAG, NEFA, total and HDL cholesterol, nor was there any significant effect on inflammatory markers, although there was a trend for higher TNF-α in the CC group at visit 2.

There were no differences between men and women in their response to the intervention.

6.5.2 Effects on glucose metabolism

Although there were overall changes in fasted and IAUC glucose between visits, there was no difference between groups. When the visits were compared within each individual group, in post hoc paired t tests, a significant difference between visits was only observed for the control group, which displayed a mean increase in fasted glucose of 0.4 mmol/L (p=0.010) and a decrease in IAUC (p=0.031). However, after adjustment was made for multiple comparisons, the difference in IAUC was no longer significant. Given that the initial analysis found no difference between groups, and that the changes in both groups were in the same direction, it seems likely that the changes in glucose parameters between visits were simply a result of repeated measurement or order effects.

This lack of effect of CC on fasted glucose is consistent with that reported by previous studies examining CC and/or caffeine, in both habitual coffee drinkers (11,12) and in coffee-naïve individuals (112,193), but in disagreement with a recent study investigating the effects of an instant green/roast coffee blend, which observed a decrease in fasted glucose following eight weeks of coffee drinking (216). They did not report a detailed
coffee analysis, but stated that their coffee contained $85.1 \pm 1.6$ mg/g total hydroxycinnamic acids, mainly CGA. Their coffee therefore contained higher amounts of CGAs and lower caffeine, providing a daily dose of 511 mg hydroxycinnamic acids and 120 mg caffeine in comparison to the coffee used in the current study which contained 174 mg total CGAs and 175 mg caffeine. The higher amount of CGAs in their coffee, may explain the reduction in fasted glucose, as *in vitro* and animal studies have reported CGAs to cause a reduction in hepatic glucose output $^{142,143}$. It should also be noted that Sarriá et al. employed a cross-over design, with more participants ($n = 52$), both of which would increase the power of their study to detect a small difference in fasted glucose.

The lack of effect of CC on postprandial glucose is consistent with studies in habitual coffee drinkers $^{11,13}$, but is in contrast to the only other study to date performed in coffee-naïve individuals, which reported a reduction in postprandial glucose after their CC intervention $^{112}$. In that study, the participants were overweight with IFG, in contrast to the current study which was a mix of normal-weight and overweight, all with normal fasted glucose. In the current study, only four participants had a baseline fasted value over 5 mmol/L, with the highest being 5.5 mmol/L, which is still within the normal range. Furthermore, Ohnaka *et al.* measured AUC, not IAUC; although they observed no significant change in fasted glucose between visits, it is still possible that individual changes in fasted values may have affected their AUC values. When individual responses in the current study were inspected, many had an increase in AUC but a decrease in IAUC or *vice versa*, demonstrating the need to take fasted glucose into account. Ohnaka *et al.* compared before and after values for individual groups and between groups without adjusting for multiple comparisons, thus increasing the probability of generating a Type I error (a false positive). As they did not report their statistics in detail, it is not possible to determine whether their differences would have remained significant after correction for multiple comparisons and indeed when they compared $\Delta$AUC for CC, DC and control by one way ANOVA they only found a statistical trend ($p=0.08$).

### 6.5.3 Effects on insulin and insulin sensitivity

In line with most other studies $^{11,13,112}$, no change in fasted insulin was observed in the CC group. This is in contrast to van Dam *et al.*, who found CC increased fasted insulin after 4 weeks, however they used a very high dose of CC in their study (equivalent to 13 cups/day) and indeed observed no significant effect on fasted insulin in a second trial, reported in the same paper, using a lower dose of approximately 10 cups/day $^{12}$. 
They reported a large number of drop-outs on the first trial due to side-effects from the high CC consumption, which led them to hypothesise that raised levels of stress hormones, such as noradrenaline, may have been implicated in the observed insulin increase. Another group, in a cross-over trial, gave either 400 mg caffeine/day or placebo for one week to a group of habitual caffeine users (mean daily pre-trial intake = 358 mg) and found caffeine raised fasted insulin \(^{(217)}\). This is the equivalent amount of caffeine to that found in 4 - 6 cups of CC, so would not be considered an excessive dose. As their participants took their last caffeine or placebo dose the day before each study day, with no extended caffeine-washout period, it is likely that their participants may not have been entirely caffeine free after the caffeine leg of the trial, as detectable levels of caffeine have previously been found after a 15 h fast \(^{(127)}\). They did not take any baseline measures before the trial, only taking blood samples at the end of each 7 day intervention period, so it is impossible to tell whether caffeine increased fasted insulin or caffeine abstention decreased it. Given that their caffeine dose was similar to their participants’ normal caffeine intake, the latter is perhaps the more likely explanation. If this is correct it would imply that long-term caffeine intake does have a hyperinsulinaemic effect and that the longer-term interventions to date have not been of a sufficient duration to reveal this effect.

The current study found no change in postprandial insulin in the CC group, in line with other longer-term CC interventions \(^{(11,13,112)}\). In contrast, a study which gave caffeine to coffee-naïve individuals for two weeks observed an increased insulin IAUC following the caffeine intervention, however, it was effectively an acute study as caffeine was also given on the day of the test \(^{(193)}\). Interestingly, in the current study, CC appeared to delay the insulin peak at the second visit, changing the time taken to reach peak insulin from 15 to 30 min. This is comparable to the reduced first-phase insulin response documented in T2DM. It is uncertain what the physiological significance of this is however, given that there was no consequent increase in glycaemia at this time, unlike in T2DM \(^{(218)}\). It should also be noted that there were no statistically significant differences between the mean insulin values at the two time-points, despite this treatment * time effect.

Fasted insulin sensitivity (HOMA2-%S), decreased unexpectedly in the control group with no change in the CC group, indicating reduced insulin sensitivity at visit 2 for the controls. This was represented by increases in both fasted glucose and insulin values in the control group.
It is possible that the unexpected observed changes in the control group were as a result of total caffeine abstention, although this is unlikely as all participants had been caffeine-free for two weeks prior to the first study day. Nevertheless, the timescale for caffeine withdrawal to impact on metabolism has not been established. Because these changes occurred in the control group rather than the intervention group, these results should be interpreted with caution, but are most likely due to random variation in glucose and insulin responses.

6.5.4 Effects on fasted lipids

No effect of CC on fasted TAG, total or HDL cholesterol was observed. This is in agreement with other interventions that have used moderate daily intakes\(^{(11,13,116)}\). However, CC has been observed to elevate both total and HDL cholesterol at a higher dose of 8 cups/day\(^{11}\). Fried et al. also compared different doses of filtered CC and observed an increase in total, LDL and HDL cholesterol in their higher-dose group (720 ml/day), but not in their lower-dose group (360 ml/day)\(^{116}\). This is somewhat consistent with the results of two meta-analyses, both of which found a dose-response effect of coffee, taken over 14 - 71 days, on total and LDL cholesterol and TAG, but not on HDL cholesterol\(^{(118,119)}\). These meta-analyses concluded that most of the effect came from studies that used boiled coffee and noted that filtered coffee had minimal effect on blood lipids. This could be explained, as boiled coffee contains high amounts of the diterpenes cafestol and kahweol, which are known to be lipid raising\(^{(114,115)}\), whereas filtered coffee contains less of these compounds as the coffee is in contact with the water for less time and the paper filter “traps” most of the lipid fraction. One of these meta-analyses suggested that caffeine may also be contributing to the effects on lipids as their subgroup analysis revealed no effect of DC\(^{(119)}\), however the other found no difference in the response between trials comparing CC with DC, concluding that caffeine has a negligible lipid-raising effect\(^{(118)}\). One study found a decrease in total and LDL cholesterol with no change to HDL cholesterol and TAG following one week of high dose instant CC drinking (24 g instant CC granules/day, equivalent to 12 cups/day), however it was poorly designed with no control for comparison so no conclusions can be drawn\(^{(219)}\).

Interestingly, the studies discussed previously which did report increased blood lipids were carried out with filtered CC, contrary to the meta-analysis results, however their coffee compositions were not reported and others have reported filtered coffee to contain large amounts of diterpenes. For example, Corrêa et al. found an increase in total and HDL cholesterol but no effect on TAG with two different roasts of filtered
CC \textsuperscript{(113)}. Their participants consumed 3 - 4 cups/day, a similar dose to that consumed in the current study, but their CC was very strong, containing 210 - 334 mg total CGA and 231 - 244 mg caffeine per cup, whereas the coffee consumed in this study contained only 44 mg CGA and 44 mg caffeine per cup; in fact the total daily dose in the current study (174 mg CGA, 175 mg caffeine) was less than one of their cups. They also reported high levels of cafestol and kahweol. These high levels of diterpenes, and possibly caffeine, may explain the lipid-raising effects observed, despite being produced by the paper filtration method.

The increase in HDL observed in these studies is interesting and may indicate some beneficial effect of coffee, however the magnitude of the increase was small (0.08 - 0.09 mmol/L) in comparison to the reported increases in total (0.24 - 0.40 mmol/L) and LDL cholesterol (0.15 - 0.17 mmol/L).

The diterpene content of the CC used in the current study was not measured, however, as it was instant coffee, it is likely to be low \textsuperscript{(220)}. It is therefore probable that the CC dose was too low in diterpenes to have had an effect on lipids. This would suggest that drinking a moderate amount of instant CC on a regular basis would not induce a detrimental effect on blood lipids.

6.5.5 Effects on postprandial TAG

The current study found no effect of CC on postprandial TAG. Although there do not appear to be any other longer-term interventions with which to compare these results, one can compare and contrast these findings with acute studies. Acute studies have demonstrated no effect of CC \textsuperscript{(221)} and coffee bean extract \textsuperscript{(222)} on postprandial TAG, although Bloomer \textit{et al.} did report a trend ($p=0.07$) for an overall difference between treatments, with CC appearing to elevate TAG at 4 h more than control \textsuperscript{(221)}, however they did not report a \textit{post hoc} analysis to confirm this.

Bloomer \textit{et al.} gave their participants a high-fat milkshake, providing 0.8 g/kg fat (which equates to 56 g for a 70 kg person), which is substantially larger than the fat content of the liquid meal consumed in the current study (23 g). Furthermore, the TAG response was measured for 2 h in the current study; as TAG typically takes 3 - 5 h to reach peak levels, only the initial rise was observed. It is possible therefore that the meal contained insufficient fat or that measures were taken over too short a time period to produce sufficient elevation in TAG for an observable effect.
6.5.6 Effects on fasted and postprandial NEFA

There was no effect of CC on fasted or postprandial NEFA in the current study. Whilst there do not appear to be any other longer-term interventions looking at the effects of CC on fasted or postprandial NEFA, there have been several acute studies.

Acute CC and caffeine ingestion have both been demonstrated to increase fasted NEFA \(^{(193,223)}\). Some have observed that this increase only occurs in normal-weight individuals and not in the obese \(^{(135)}\), however it should be noted that fasted levels in the obese were typically much higher and the elevation observed in the normal-weight group resulted in levels comparable to the baseline levels found in the obese. Daubresse et al. also noted that DC had no effect on fasted NEFA in either group, suggesting that caffeine is responsible for the effect \(^{(135)}\). A degree of acclimatisation to these effects of caffeine seems to occur as trials comparing the effects of caffeine on NEFA levels before and during exercise found that acute caffeine ingestion increased NEFA levels more in caffeine-naïve individuals than in regular caffeine users \(^{(224,225)}\). Also, Dekker et al. observed that an acute dose of caffeine resulted in less of an increase in NEFA and adrenalin levels after 14 days of caffeine use in previously caffeine-naïve individuals \(^{(193)}\). Fisher et al. also found caffeine caused less of an increase in adrenalin in the habitual caffeine users \(^{(225)}\), which may explain the reduced NEFA increase as adrenalin stimulates adipose tissue lipolysis through its effects on cAMP and HSL.

When postprandial studies are examined, results are more inconsistent. One study found caffeine, but not CC, elevated fasted NEFA more than control in the hour preceding an OGTT, but noted that levels were not different between treatments by the end of the OGTT \(^{(10)}\). They also noted that DC reduced the overall NEFA response compared to both caffeine and control, observing greater postprandial NEFA suppression following DC consumption than that produced by both caffeine and placebo, suggesting that components other than caffeine may oppose the effects of caffeine. Another study found that CC had no effect on NEFA for the first 2 h after a mixed meal, but elevated NEFA above control levels in the third hour \(^{(8)}\). In this case insulin had returned to near fasting levels and so would have a reduced ability to suppress NEFA, whereas it is likely that there was still a substantial amount of caffeine in the blood continuing to elevate NEFA compared to control.

When caffeine was infused intravenously at a steady rate of 0.6 mg/kg/h (15 min after a priming dose of 3 mg/kg) during a hyperinsulinaemic-euglycaemic clamp procedure, caffeine was found to increase fasted NEFA more than control and to maintain
elevated NEFA levels over the entire 2 h time-course of the study \(^{(226)}\). This apparently stronger effect of caffeine is consistent with that observed by Battram \textit{et al.} and gives further weight to the argument that non-caffeine components of coffee may be attenuating the acute effects of caffeine.

A high intra-individual variation in fasted NEFA has been demonstrated, with one study reporting a CV of 45\% in individuals measured on successive mornings \(^{(51)}\), although others have found lower variation over longer periods; Magkos \textit{et al.} reported a CV of 24\% and concluded that 6 - 10 participants in a crossover study and 12 - 20 per group in a parallel-arm design would be sufficient to detect differences of 25 - 30\% in fasted NEFA \(^{(227)}\). As there were 19 participants in the coffee group and only 7 in the control in the current study, it is quite plausible that it was underpowered with respect to NEFA measurement.

Perhaps more importantly, however, the major difference between this study and the aforementioned acute studies is the absence of CC immediately prior to the meal. This means that the participants in the current study had no caffeine or other coffee components in their blood at the time of meal consumption.

6.5.7 Effects on inflammatory markers

The link between coffee and inflammatory markers is unclear. Some epidemiological studies suggest an association between coffee drinking and raised levels of both IL-6 and TNF-\(\alpha\) \(^{(105,228)}\), some have found no association with IL-6 \(^{(102)}\) and others have found lower levels of TNF-\(\alpha\) in coffee drinkers \(^{(106)}\). A recent meta-analysis of 10 prospective cohorts found an association between IL-6 and T2DM risk \(^{(54)}\). Animal models have demonstrated a reduction in TNF-\(\alpha\) in rats, following 8 weeks of DC consumption \(^{(229)}\), and a reduction in IL-6 and TNF-\(\alpha\) in mice following 5 weeks of CC consumption \(^{(230)}\), with both studies giving coffee doses equivalent to normal coffee consumption in humans. Longer-term interventions in humans however have produced contradictory results, with some finding no effect of CC on these markers \(^{(11,113)}\) whilst others have observed an increase in IL-6 \(^{(13)}\), reflecting the ambiguous results in epidemiological data.

Several of coffee’s key components have exhibited both antioxidant and anti-inflammatory effects \textit{in vitro}, including caffeine \(^{(231)}\), CGAs \(^{(232)}\), cafestol and kahweol \(^{(233,234)}\), whilst a temporary increase in antioxidant capacity following acute coffee drinking has been demonstrated \textit{in vivo} \(^{(235)}\). This has led to suggestions that coffee may exert a beneficial effect on health via antioxidant and anti-inflammatory
effects. Dietary antioxidants however are present in far smaller quantities than their endogenous counterparts and it is debatable as to how large a contribution they make to the overall antioxidant activity in the body.

Consistent with 2/3 of the previous human interventions, the current study found no significant effect of coffee on TNF-α and IL-6. There was however a trend for an increase in TNF-α at visit 2 within the CC arm, which may be indicative of a real increase. The CC was relatively low in caffeine and total CGAs, and as noted previously, likely to be low in the diterpenes; it is possible therefore that it simply did not contain sufficient antioxidants to initiate a detectable change.

Plasma IL-6 levels are known to vary from day to day and can be influenced by a range of factors including diet and menstrual cycle stage (236). The current study did not control the previous day’s diet, apart from the evening meal, nor was there any control of menstrual cycle, although as the intervention lasted 12 weeks it is likely that the female participants were in the same stage at each visit, provided they had a regular cycle. It is possible however that this lack of stricter controls contributed to greater variation in response.

When individual responses were examined, large inter-individual variation was apparent with almost half of each group exhibiting an increase between visits and half exhibiting a decrease, for both IL-6 and TNF-α. Under these circumstances it is unlikely that the lack of effect was simply due to study power.

6.5.8 Gender differences

Cross-sectional studies have reported IGT to be more common in women and IFG to be more prevalent in men (237,238). Whilst none of the participants had IFG, higher fasted glucose was observed in the males, perhaps indicating greater susceptibility to development of IFG in later life. It has been suggested that gender differences in IGT, as measured by 2 h glucose load, are attributable to height differences as taller people, usually men, have more muscle mass and thus higher rates of glucose disposal (238). Interestingly, no correlation between gender and height was found in the current study (Pearson’s correlation, p=0.142) and there was no difference in the 2 h glucose values. There was however a trend for a lower overall glucose response in women, in contrast to Couillard et al., who reported a lower glucose IAUC in men along with higher fasted glucose and higher postprandial insulin (210). However, when their participants were matched for abdominal visceral adipose tissue, the difference in postprandial insulin and glucose disappeared, leading the authors to conclude that increased abdominal
adiposity in men may be the cause of the gender differences. Abdominal adiposity was
not measured in the current study, so it is unknown whether this was a factor in the
results.

Gender differences in lipid metabolism have also been observed, with men generally
having higher fasted TAG, lower HDL and higher total and LDL cholesterol than
women \(^{(210,211)}\). Men have also been reported to have higher postprandial TAG and
NEFA, although the difference in NEFA was only apparent more than 2 h after the test
meal \(^{(210)}\). Others have also observed greater NEFA suppression in women than in
men \(^{(212,239)}\). When the participants in Couillard et al.’s study were matched for
abdominal visceral adipose tissue, the difference in postprandial TAG disappeared,
but the difference in NEFA remained. Changes in adiposity have also been correlated
with changes in fasted cholesterol, HDL and TAG \(^{(240)}\). Others have observed no
difference in fasted or postprandial TAG between men and women following a large
lipid load, despite finding significant differences between genders for height and body
fat \(^{(241)}\).

Contrary to the larger cohort studies, the current study did not find any significant
gender differences in fasted TAG (males: mean 1.03 mmol/L, SD 0.45; females: mean
0.89 mmol/L, SD 0.29), total cholesterol (males: mean 4.03 mmol/L, SD 0.58; females:
mean 4.20 mmol/L, SD 0.62) or HDL cholesterol (males: mean 1.22 mmol/L, SD 0.23;
females: mean 1.33 mmol/L, SD 0.25). The participants were all healthy with few (<=2)
having levels outside the healthy range for any individual measure; this perhaps
explains the lack of difference between genders. A trend was observed, however, for
higher postprandial TAG in men and greater NEFA suppression in women in line with
previous studies. It has been suggested that differences in postprandial TAG between
men and women may be explained by enhanced skeletal muscle TAG clearance in
women \(^{(242)}\). Interestingly the men in the current study displayed minimal NEFA
suppression, similar to what may be observed in T2DM, which was an unexpected
finding.

Differences in inflammatory markers have also been observed, with women having
been reported to have lower TNF-\(\alpha\) and a stronger correlation between adiposity and
IL-6 than men \(^{(243)}\). Unlike the epidemiology, there was no significant difference
between men and women for either IL-6 or TNF-\(\alpha\) in the current study, however a slight
trend for lower TNF-\(\alpha\) was observed in women. This lack of significant effect is possibly
due to the high inter-individual variation previously described or may simply be that the
population was too healthy for small differences to be detected with the study power.
Epidemiology has variously demonstrated a reduced risk of T2DM with coffee drinking in men, but not in women (213), a reduced risk for both (3) and a reduced risk in women with only a slight trend in men (214). Few studies have compared gender responses to the acute effects of coffee on the postprandial glucose and insulin response, with these reporting mixed findings. One study found women to have a greater increase in postprandial glucose than men and men to have a reduction in peak insulin following acute CC consumption (184), whereas another found no difference between genders when an acute caffeine dose was given (195). None of the existing interventions investigating CC’s longer-term effects on glucose metabolism have compared gender responses.

A meta-analysis of longer-term interventions examining the effect of coffee on fasted lipids found that the effects of coffee were significantly higher in those studies that included women (119), suggesting perhaps that women are more susceptible to the effects that coffee has on fasted lipids, however no direct comparisons between men and women were made.

Adding gender as a cofactor to the analysis in the current study did not materially affect any results, with no differences observed between men and women in their response to CC for any variable. This is perhaps unexpected given that some epidemiology and acute studies have observed gender differences, however, the evidence is limited and contradictory and direct comparison cannot be made between a longer-term intervention and acute studies and cross-sectional analyses.

### 6.5.9 Potential confounders

It has previously been established that oral contraceptive use affects caffeine metabolism, with some studies demonstrating an almost doubling of the half-life from 3 - 5 h to 6 - 10 h (244–246). Oral contraceptive use was not an exclusion criteria for this study, however only two of the 15 women recruited to the study were taking oral contraceptives, so this is unlikely to have been a significant confounder.

There is also conflicting evidence as to whether age affects postprandial glucose metabolism, with some authors finding an effect of age (247,248) and others suggesting little or no effect after adjustment for obesity/bodyweight and/or fitness levels (249,250). One study found that an effect of age remained after these adjustments, but in women only (251). As there was no significant difference between CC and control groups for age it is unlikely to have been a significant confounder in this study.
Caffeine withdrawal symptoms, such as headache, tiredness and irritability, typically appear within 12 – 24 h of caffeine abstinence, peak within 20 – 51 h and can last from 2 – 9 d \(^{(252)}\). The participants in the CC group had been caffeine-free for approximately 64 h on their second study visit, so are likely to have passed the time of peak withdrawal symptoms, but may have still been suffering from some unrecognised symptoms. All were asked if they thought they had suffered from caffeine withdrawal, with most reporting some level of tiredness or headache on the day immediately following their last coffee dose. Several reported still feeling tired on the study day, but none reported any residual headache. A greater NEFA response to acute caffeine ingestion has been observed following four days of caffeine withdrawal in comparison with a control group who had not experienced caffeine withdrawal \(^{(253)}\), however this NEFA rise was in response to caffeine and no caffeine was given on the study days in the current study.

The evening meal on the day prior to each study day was kept the same as food taken on the evening prior to an OGTT has been demonstrated to have a carry-over effect \(^{(254)}\); a high-fat evening meal (62% energy from fat, 31% energy from CHO) was observed to result in lower fasted TAG and a greater postprandial glucose and TAG response to an OGTT the following morning, compared to a high CHO meal (16% energy from fat, 76% energy from CHO). The evening meal for the current study contained 40% energy from fat and 41% from CHO, which is much lower than the high fat meal used by Robertson et al. \(^{(254)}\).

Genetic variability may also be confounding the results. Several SNPs in the ADORA2 gene, which codes for the adenosine receptor A\(_{2b}\)R, are associated with IL-6 and CRP levels \(^{(255)}\). The Nco-1 polymorphism in the TNF-\(\alpha\) gene is associated with higher TNF-\(\alpha\) concentrations, greater insulin AUC and reduced insulin sensitivity \(^{(256)}\). As the participants were not genotyped for these polymorphisms, these are potential confounders, particularly as when individual responses were examined (data not shown) the variation in response to the intervention was very diverse.

Due to the complex and intertwining nature of glucose and lipid regulation, it is impossible to rule out the confounding of results by other factors. For example, correlations exist between fasted NEFA and BMI, fasted glucose, glucose AUC and fasted TAG \(^{(48)}\). With such a small study population, it was not possible to control for all these factors or advisable to include them in the analysis as covariates.
6.5.10 Weaknesses

There is a possibility of order effects in the current study as this was not a crossover trial, however there was a control group. Ideally it would have been designed as a crossover intervention, however, because the aim was to examine the effects of CC on coffee-naïve individuals, this was not possible; as it is not known how long any effects of CC would take to be reversed, a suitable washout period could not be accurately determined. The order effect could have been minimised by including a dummy study day at the start of the intervention to allow participants to become familiar with the protocol and reduce any effects that may have resulted from stress during the first visit. This is something that should be considered for future work and has become routine for some interventions within the group. Duplicate study visits could also be considered for the future in order to reduce intra-individual variation, but this would have a time, cost and study burden implication for participants.

The study population was very heterogeneous with marked individual variability. Whilst this makes the results more applicable to the general population it also reduces the power of the study to find between-group differences. It is possible that larger differences would have been detectable in a more homogeneous population.

6.6 General discussion/conclusion

To our knowledge, this is only the second study to examine the longer-term effects of CC drinking in coffee-naïve individuals, and the first to examine those effects in healthy people with apparently normal glucose metabolism. Furthermore it appears to be the first to investigate gender differences in postprandial glucose and lipid metabolism in response to longer-term CC consumption.

Contrary to the hypothesis that longer-term coffee drinking would reduce the postprandial glycaemic response, it would appear from these results that regular moderate consumption of instant CC does not affect fasted or postprandial glucose or lipid metabolism in healthy adults. It may however cause a delayed insulin peak. These results are in contrast to the only other study that has been performed in coffee-naïve individuals, which found a reduction in glucose response following 16 weeks of instant CC consumption (112). Compliance cannot explain the difference in results as it was comparable in both trials; median salivary caffeine concentrations in the current study were 8.9 µM in the coffee group and 0.1 µM in the control group, whereas Ohnaka et al. reported median concentrations of 6.9 - 8.2 µM in their coffee group. There are however several differences between the two protocols. Ohnaka et al. studied
overweight, older men, who already had IFG, whereas the current study group were generally normal weight and younger with healthy fasted glucose. It is perhaps not possible to see an improvement in a group that is already very healthy.

It is possible that an intervention in younger healthy adults would have to be extended over a much longer time-frame than 12 weeks to see an effect, as it could be hypothesised that coffee exerts its effects by preventing/attenuating the decline in health status which occurs over time, rather than causing a reduction in any of these parameters. If this is the case then an intervention may need to last for several years for the true effects to become evident.

It should be noted, however, that the CC consumed in this study was relatively low in caffeine and CGAs and likely also low in diterpenes. Since these are the components that have been suggested to be responsible for the effects of coffee, it is possible that it did not contain sufficient quantities of these components to produce a significant effect on any of the measured parameters.

Given the amount of variation in response between participants, particularly in the CC group, it is possible that some other unknown factor is interacting with the effects of coffee. One such possibility is explored further in Chapter 7.

Finally, it should be acknowledged that the study design does not reflect a real world scenario, as participants were tested after a two day washout period and regular coffee drinkers are unlikely to abstain from coffee for this period of time and are indeed likely to have some level of caffeine in their system for most of the day. Nevertheless, the results are valid in that they reflect the effects of longer-term CC consumption, without being compromised by the known acute effects of CC and caffeine.

In conclusion, this study found no effects on glucose and lipid metabolism following moderate instant CC consumption in healthy adults. Combined with the limited literature, there is insufficient evidence to make a definitive statement as to whether CC drinking has a detrimental or beneficial effect at this stage. There is clearly a need for further research, perhaps over a longer time period with a larger sample size and different study populations.
Chapter 7. The effects of 12 weeks of coffee drinking on glucose and lipid metabolism in coffee-naïve individuals: secondary analysis

7.1 Introduction

The results of previous coffee studies may be confounded by individual differences in caffeine metabolism. As discussed in the introduction to this thesis (Chapter 1), a particular SNP in the CYP1A2 gene, rs762551, has been demonstrated to affect caffeine metabolism (198), with carriers of the C allele being classed as being of a slow metaboliser phenotype whereas those who are homozygous for the A allele are considered to be of a fast metaboliser phenotype. Case-control and prospective cohort studies have reported an association between coffee drinking and increased risk of myocardial infarction (257), hypertension (258,259) and IFG (160) in those who are slow caffeine metabolisers but not in fast metabolisers.

It is possible that these polymorphisms in the CYP1A2 gene will modulate the effects of coffee on glucose and lipid metabolism however, to date, no acute or longer-term interventions have investigated this. The secondary aim of the 12 week intervention, detailed in Chapter 6, was to examine whether there were any differences between fast and slow metaboliser phenotypes. This chapter reports on the outcome of this secondary analysis.

7.2 Aims

To examine whether there were any differences in glucose and lipid metabolism between fast and slow phenotypes in all participants at baseline.

To examine whether there were any differences in the effects of the intervention on these measures between slow and fast phenotypes in the coffee group.

To further examine whether there were any gender-phenotype interactions.

7.3 Methods

The methods and protocol are as detailed in Chapter 6.
7.3.1 Statistical analysis

All statistical analysis was carried out using SPSS Statistics software, version 22. The Shapiro-Wilks test was used to check normality of data. Two-way RM ANOVA was used to compare groups over time-points. Student’s t tests were used for comparisons between groups when the data were normally distributed and the equivalent non-parametric tests were used when they were not. Fisher’s Exact tests were used for assessment of independence of variables. Statistical significance was classed as a p-value < 0.05.

7.4 Results

This section will report each measure in turn. For each measure, the results from the analysis by phenotype, of baseline measures in the whole cohort (n=27) will be reported first, followed by the results from the comparison of phenotypes in the coffee group (n=19) at baseline and after the intervention. The control group was excluded from this second part of the analysis because, as a control, changes were not anticipated in this group. Furthermore, as numbers in the control group were low (n=8), the study was underpowered to perform this analysis in this group.

The results are summarised in tables in Section 7.4.3.

7.4.1 Baseline participant characteristics

All participants (n=27)

There were no statistically significant differences between the slow and fast metaboliser phenotypes at baseline for any of the anthropometric measures when the CC and control groups were combined, although there was a trend for those with the fast phenotype to be younger (p=0.063) with higher SBP (p=0.075). When males and females were examined separately and the two phenotypes were compared, male slow metabolisers had a higher fasted glucose (p=0.022) and there was a trend for male slow metabolisers to have a higher body fat percentage (p=0.050) and to be older (p=0.070) than male fast metabolisers; there were no differences for any of the tested parameters between female slow and fast metabolisers (p>0.129). The baseline participant characteristics, split by phenotype, are reported in Table 7.4.1-1.
Coffee group at baseline (n=19)

There were no statistically significant differences between the slow and fast metaboliser phenotypes in the CC group at baseline for any of the anthropometric measures (p>0.218). The majority of females were slow metabolisers (67%), whereas most males were fast metabolisers (70%), but this difference in the proportion of fast to slow metabolisers between males and females was not statistically significant (Fisher’s Exact, p=0.179). When males and females were examined separately and the two phenotypes were compared, male slow metabolisers had a higher body fat percentage (p=0.039) and a trend for higher weight (p=0.079) and higher BMI (p=0.064) than male fast metabolisers; there were no differences for any of the tested parameters between female slow and fast metabolisers (p>0.173).

When age was analysed by two-way RM ANOVA there was a trend for an effect of gender (p=0.053), phenotype (p=0.053) and a phenotype * gender interaction (p=0.053). Examination of the marginal means revealed that slow males (mean 32 y; 95% CI: 25.9, 37.4) were older than the other gender/phenotype combinations (fast males: mean 22 y; 95% CI: 18.2, 25.8; fast females: mean 22 y; 95% CI: 16.3, 27.7; slow females: mean 22 y; 95% CI: 17.9, 26.1).

Similarly, when body fat percentage was analysed by two-way RM ANOVA, a phenotype * gender interaction was found (p=0.013), along with a trend for an effect of gender (p=0.057). Examination of the marginal means revealed fast males to have the lowest body fat percentage (mean 15.0%; 95% CI: 10.1, 19.9), followed by slow females (mean 23.1%; 95% CI: 17.8, 28.4) and slow males (mean 25.3%; 95% CI: 17.8, 32.8), with fast females having the highest body fat percentage (mean 29.7%; 95% CI: 22.2, 37.2).

Table 7.4.1-1 Baseline characteristics for all participants, by phenotype and gender.

<table>
<thead>
<tr>
<th></th>
<th>Fast phenotype</th>
<th>Slow phenotype</th>
<th>All (n=13)</th>
<th>Fast phenotype</th>
<th>Slow phenotype</th>
<th>All (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female (n=4)</td>
<td>Male (n=9)</td>
<td>All (n=13)</td>
<td>Female (n=9)</td>
<td>Male (n=5)</td>
<td>All (n=14)</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
</tr>
<tr>
<td>Age at start (y)</td>
<td>23.3 (3.6)</td>
<td>22.2 (2.8)</td>
<td>22.3 (3.0)</td>
<td>23.4 (1.1)</td>
<td>32.6 (9.6)</td>
<td>26.7 (7.6)</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.69 (0.06)</td>
<td>1.69 (0.11)</td>
<td>1.69 (0.10)</td>
<td>1.54 (0.08)</td>
<td>1.74 (0.04)</td>
<td>1.68 (0.08)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>63.1 (5.2)</td>
<td>67.2 (11.1)</td>
<td>65.9 (9.6)</td>
<td>60.7 (15.0)</td>
<td>77.5 (13.1)</td>
<td>66.7 (16.2)</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>120 (9.9)</td>
<td>123 (9.4)</td>
<td>122 (9.3)</td>
<td>112 (6.3)</td>
<td>121 (11.3)</td>
<td>116 (9.1)</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>71 (6.4)</td>
<td>71 (7.4)</td>
<td>71 (6.8)</td>
<td>73 (7.9)</td>
<td>74 (8.7)</td>
<td>74 (7.9)</td>
</tr>
<tr>
<td>BMI (kg/m^2)</td>
<td>22.1 (0.8)</td>
<td>23.3 (1.8)</td>
<td>22.9 (1.6)</td>
<td>22.2 (3.3)</td>
<td>25.7 (4.3)</td>
<td>23.5 (3.9)</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>29.9 (4.7)</td>
<td>14.2 (4.4)</td>
<td>19 (8.7)</td>
<td>25 (8.5)</td>
<td>21.7 (8.3)</td>
<td>23.8 (8.3)</td>
</tr>
<tr>
<td>Fasted glucose (mmol/L)</td>
<td>4.2 (0.4)</td>
<td>4.6 (0.2)</td>
<td>4.5 (0.4)</td>
<td>4.6 (0.4)</td>
<td>5.0 (0.3)</td>
<td>4.7 (0.4)</td>
</tr>
<tr>
<td>Fasted insulin (pmol/L)</td>
<td>65 (23)</td>
<td>61 (21)</td>
<td>62 (21)</td>
<td>61 (22)</td>
<td>72 (24)</td>
<td>65 (22)</td>
</tr>
</tbody>
</table>

* Trend for a difference between slow and fast phenotypes (p<0.10). ¥ Trend for a difference between male slow and fast phenotypes (p<0.10). ¥¥ Significant difference between male slow and fast phenotypes (p<0.05). BMI: body mass index; BP: blood pressure; SD: standard deviation.
The baseline participant characteristics for the CC group, split by phenotype, are reported in Table 7.4.1-2.

Table 7.4.1-2 Baseline characteristics, within the coffee group, by phenotype and gender.

<table>
<thead>
<tr>
<th></th>
<th>Fast phenotype</th>
<th></th>
<th>Slow phenotype</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female (n=3)</td>
<td>Male (n=7)</td>
<td>All (n=10)</td>
<td>Female (n=6)</td>
</tr>
<tr>
<td>Age at start (y)</td>
<td>Mean 22 SD 3.6</td>
<td>Mean 22 SD 3.0</td>
<td>Mean 22 SD 3.0</td>
<td>Mean 22 SD 2.3</td>
</tr>
<tr>
<td>Height (m)</td>
<td>Mean 1.68 SD 0.07</td>
<td>Mean 1.73 SD 0.10</td>
<td>Mean 1.71 SD 0.09</td>
<td>Mean 1.62 SD 0.03</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>Mean 62.9 SD 6.4</td>
<td>Mean 70.5 SD 10.0</td>
<td>Mean 68.3 SD 9.4</td>
<td>Mean 56.5 SD 8.3</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>Mean 115 SD 5.3</td>
<td>Mean 124 SD 10.6</td>
<td>Mean 121 SD 10.0</td>
<td>Mean 113 SD 7.0</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>Mean 72 SD 7.4</td>
<td>Mean 71 SD 7.4</td>
<td>Mean 71 SD 7.0</td>
<td>Mean 72 SD 3.4</td>
</tr>
<tr>
<td>BMI (kgm⁻²)</td>
<td>Mean 22.3 SD 0.8</td>
<td>Mean 23.6 SD 1.9</td>
<td>Mean 23.2 SD 1.7</td>
<td>Mean 21.6 SD 2.7</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>Mean 29.7 SD 5.8</td>
<td>Mean 15 SD 4.8</td>
<td>Mean 19.4 SD 8.5</td>
<td>Mean 23.1 SD 6.3</td>
</tr>
<tr>
<td>Fasted glucose (mmol/L)</td>
<td>Mean 4.2 SD 0.5</td>
<td>Mean 4.7 SD 0.2</td>
<td>Mean 4.5 SD 0.4</td>
<td>Mean 4.6 SD 0.6</td>
</tr>
<tr>
<td>Fasted insulin (pmol/L)</td>
<td>Mean 67 SD 27</td>
<td>Mean 63 SD 24</td>
<td>Mean 64 SD 23</td>
<td>Mean 64 SD 25</td>
</tr>
</tbody>
</table>

¥ Trend for a difference between male slow and fast phenotypes (p<0.10). ¥¥ Significant difference between male slow and fast phenotypes (p<0.05). BMI: body mass index; BP: blood pressure; SD: standard deviation.

Pre-intervention coffee and caffeine intake

Pre-intervention coffee and caffeine intake were estimated as described in Chapter 2; there were no significant differences between fast and slow metabolisers for either caffeine or coffee intake (Mann-Whitney U, p>0.549).

7.4.2 Effects of coffee intervention: comparison of fast and slow metabolisers

7.4.2.1 Anthropometrics

Weight, body fat, BMI, SBP and DBP were measured at each of the two study visits. Paired samples t tests were run for each phenotype to establish whether there were any changes between the two visits; no within group differences were found for any measure (p>0.180). All anthropometrics were further analysed by RM ANOVA for any differences in the changes between the two phenotypes; there were no between group differences for any measure (p>0.097). Nor were there any differences between visits for any measure when the male and female, fast and slow phenotype subgroups were analysed independently (data not show, paired t test, p>0.155). The changes between visits for each phenotype are reported in Table 7.4.2.1-1.
Baseline data: all participants (n=27)

When the baseline glucose data for all participants was analysed by phenotype there were no differences found between fast and slow phenotypes for fasted glucose (unpaired t test, p=0.141) or IAUC (unpaired t test, p=0.524), nor was there any phenotype * time interaction (RM ANOVA, p=0.442). There was, however, a difference at 90 min (unpaired t test, p=0.040), with the slow phenotype having a higher mean glucose at this time-point. The 2 h postprandial glucose response for each phenotype is shown in Figure 7.4.2.2-1.

Table 7.4.2.1-1 Changes in anthropometric measures between the two study visits by phenotype within the coffee group.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Mean change</th>
<th>SD</th>
<th>Mean change</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast (n=10)</td>
<td>Weight (kg)</td>
<td>0.1</td>
<td>0.8</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>BMI (kgm⁻²)</td>
<td>0.0</td>
<td>0.3</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>Body fat (%)</td>
<td>0.1</td>
<td>1.1</td>
<td>-0.1</td>
</tr>
<tr>
<td></td>
<td>Systolic BP (mmHg)</td>
<td>0.9</td>
<td>7.1</td>
<td>-1.2</td>
</tr>
<tr>
<td></td>
<td>Diastolic BP (mmHg)</td>
<td>2.4</td>
<td>7.7</td>
<td>-3.8</td>
</tr>
<tr>
<td>Slow (n=9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

BMI: body mass index; BP: blood pressure; SD: standard deviation.

7.4.2.2 Plasma glucose

Baseline data: all participants (n=27)

When the baseline glucose data for all participants was analysed by phenotype there were no differences found between fast and slow phenotypes for fasted glucose (unpaired t test, p=0.141) or IAUC (unpaired t test, p=0.524), nor was there any phenotype * time interaction (RM ANOVA, p=0.442). There was, however, a difference at 90 min (unpaired t test, p=0.040), with the slow phenotype having a higher mean glucose at this time-point. The 2 h postprandial glucose response for each phenotype is shown in Figure 7.4.2.2-1.
The baseline glucose data were further analysed by two-way ANOVA with gender and phenotype as cofactors; an effect of gender (p<0.014) and of phenotype (p<0.019) was found for both fasted glucose and glucose time-point data, but there was no gender * phenotype interaction (p>0.203). Examination of the data revealed that females and those with the fast metaboliser phenotype had lower fasted glucose and a lower overall glucose response. Post hoc analysis comparing the glucose time-point data for phenotypes within gender (one-way RM ANOVA) revealed a difference in glucose response between fast and slow phenotypes for females only (p=0.009), with no significant difference in males (p=0.580). The 2 h glucose response for all participants, split by gender and phenotype is shown in Figure 7.4.2.2-2.

[Figure 7.4.2.2-2 Baseline postprandial glucose response for all participants, by gender and phenotype.]

There was a significant difference between genders and between phenotypes for fasted glucose (two-way ANOVA, p<0.014) and for overall glucose response (two-way RM ANOVA, p<0.019). Error bars are SEM (fast females: n=4; fast males: n=9; slow females: n=9; slow males: n=5).

**Effect of phenotype on plasma glucose in the coffee group (n=19)**

There was no difference in fasted glucose between fast and slow phenotypes in the CC group at baseline (unpaired t test, p=0.300). Baseline data were further analysed by two-way ANOVA, with gender and phenotype as cofactors (data not shown). An effect of gender (p=0.041) and a trend for an effect of phenotype (p=0.075) was observed for fasted glucose and an effect of gender and of phenotype (p<0.020) was
observed for the overall baseline postprandial glucose response, similar to that observed when all participants were included in the analysis.

There were no within or between group differences in the change in fasted glucose between visits for fast and slow phenotypes (RM ANOVA, p>0.135). This did not change significantly when gender was added as a cofactor.

Repeated measures ANOVA on IAUC data found an effect of visit (p=0.049) and a visit * phenotype effect (p=0.024), with the fast phenotype exhibiting a slight increase in IAUC between visits and the slow phenotype displaying a decrease between visits. When time-point data were analysed by RM ANOVA there was a visit * phenotype effect (p=0.048), with the CC intervention resulting in an apparent overall increase in glucose response in the fast phenotype group and a decrease in the slow phenotype group. The 2 h postprandial glucose response at each visit for each phenotype in the CC group is shown in Figure 7.4.2.2-3.

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**Figure 7.4.2.2-3** Postprandial glucose response by visit and phenotype, within the coffee group. Panel A: slow; Panel B: fast. The effect of coffee varied by phenotype (RM ANOVA, p=0.048). Error bars are SEM (fast: n=10; slow: n=9).
Adding gender as a cofactor to the between-visits analysis did not affect these results; the visit * phenotype effect remained significant (p=0.035) and an effect of gender was also observed (p=0.012), with females having a lower overall glucose response than males. *Post hoc* analysis was employed to determine the source of these differences. When genders were examined individually (one-way RM ANOVA) there was a trend for a visit * phenotype effect (p=0.067) for females only (panels C and D, Figure 7.4.2.2-4), with no significant difference in males (p=0.419). *Post hoc* analysis, examining the phenotypes separately (one-way RM ANOVA) found a difference between visits (p=0.031) and an effect of gender (p=0.018) for the fast phenotype only (panels B and D, Figure 7.4.2.2-4), with no significant difference between visits (p=0.400) or genders (p=0.214) in the slow phenotype.

![Figure 7.4.2.2-4 Postprandial glucose response by visit, phenotype and gender, within the coffee group. Panel A: males with slow phenotype; Panel B: males with fast phenotype; Panel C: females with slow phenotype; Panel D: females with fast phenotype. The effect of coffee varied by phenotype (Two-way RM ANOVA, p=0.035) and there was an overall effect of gender (p=0.012). Error bars are SEM (fast females: n=3; fast males: n=7; slow females: n=6; slow males: n=3).](image-url)
7.4.2.3 Serum insulin

Baseline data: all participants (n=27)

When baseline data for the whole cohort were analysed there were no differences found between fast and slow phenotypes for fasted insulin (unpaired t test, p=0.803) or IAUC (unpaired t test, p=0.139), nor was there any phenotype * time effect (RM ANOVA, p=0.340), however there was a difference between fast and slow phenotypes at 90 min (unpaired t test, p=0.020). Adding gender as a cofactor did not significantly affect these results (data not shown). The 2 h postprandial insulin response for each phenotype is shown in Figure 7.4.2.3-1.

![Figure 7.4.2.3-1 Baseline postprandial insulin response for all participants, by phenotype. There were no significant differences between phenotypes either for fasted insulin or IAUC (unpaired t test, p>0.138). Error bars are SEM (fast: n=13; slow: n=14).](image)

Effect of phenotype in the coffee group (n=19)

There was no difference in fasted serum insulin or IAUC between fast and slow phenotypes in the CC group either at baseline (unpaired t test, p>0.103) or between visits (RM ANOVA, p>0.353). There was a difference between fast and slow phenotypes at 90 min for the baseline visit (p=0.009), in line with that found for all participants.
Repeated measures ANOVA on time-point data found a visit * time effect (p=0.019), but no effect of phenotype, with both phenotypes having a delayed peak (60 min) at the second visit. Again, adding gender as a cofactor did not significantly affect these results (data not shown). The 2 h postprandial insulin response by visit for each phenotype in the CC group is shown in Figure 7.4.2.3-2.

Figure 7.4.2.3-2 Postprandial insulin response by visit and phenotype, within the coffee group. Panel A: slow; Panel B: fast. A visit * time effect (RM ANOVA, p=0.019) was found, but no effect of phenotype, with both phenotypes having a delayed peak at visit 2 (60 min). Error bars are SEM (fast: n=10; slow: n=9).
Chapter 7.

7.4.2.4 Measures of insulin sensitivity and beta cell function

**Baseline data: all participants (n=27)**

There were no differences at baseline between fast and slow phenotypes in the whole cohort for any measure (unpaired t test, p>0.146). When the analysis was repeated with phenotype and gender as cofactors (two-way ANOVA), there was a trend for an effect of phenotype on the Matsuda index (p=0.084), with the fast phenotype having a slightly higher Matsuda index (mean 6.62; SD 2.8) than the slow phenotype (mean 5.25; SD 1.8) Baseline data for all participants, split by phenotype are reported in Table 7.4.2.4-1. There was also an effect of gender on the Matsuda*AUC_{I/G} product (p=0.044), with the females having a higher product (mean 442; SD 153) than the males (mean 349; SD 64).

**Table 7.4.2.4-1 Baseline insulin sensitivity measures, for all participants, by phenotype.**

<table>
<thead>
<tr>
<th></th>
<th>Slow (n=14)</th>
<th>Fast (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>HOMA-2 %S</td>
<td>83.02</td>
<td>29.95</td>
</tr>
<tr>
<td>HOMA-2 %B</td>
<td>131.04</td>
<td>20.93</td>
</tr>
<tr>
<td>Matsuda</td>
<td>5.25</td>
<td>1.78</td>
</tr>
<tr>
<td>AUC_{I/G}</td>
<td>80.99</td>
<td>29.34</td>
</tr>
<tr>
<td>Matsuda*AUC_{I/G}</td>
<td>388.93</td>
<td>128.81</td>
</tr>
</tbody>
</table>

HOMA: Homeostatic model of assessment; %S: sensitivity; %B: beta cell function; AUC_{I/G}: ratio of insulin to glucose area under curve; Matsuda * AUC_{I/G}: product of Matsuda index and AUC_{I/G} ratio.

**Effect of phenotype in coffee group (n=19)**

There was no effect of phenotype in the CC group either at baseline (unpaired t test p>0.133) or between visits (RM ANOVA, p>0.225) for any measure. Mean values by visit for each phenotype are reported in Table 7.4.2.4-2.

**Table 7.4.2.4-2 Insulin sensitivity measures by visit and phenotype, within the coffee group.**

<table>
<thead>
<tr>
<th></th>
<th>Slow (n=9)</th>
<th>Fast (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Visit 1</td>
<td>Visit 2</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>HOMA-2 %S</td>
<td>77.77</td>
<td>31.51</td>
</tr>
<tr>
<td>HOMA-2 %B</td>
<td>136.57</td>
<td>18.07</td>
</tr>
<tr>
<td>Matsuda</td>
<td>4.76</td>
<td>2.02</td>
</tr>
<tr>
<td>AUC_{I/G}</td>
<td>89.32</td>
<td>28.33</td>
</tr>
<tr>
<td>Matsuda*AUC_{I/G}</td>
<td>383.31</td>
<td>120.42</td>
</tr>
</tbody>
</table>

HOMA: Homeostatic model of assessment; %S: sensitivity; %B: beta cell function; AUC_{I/G}: ratio of insulin to glucose area under curve; Matsuda * AUC_{I/G}: product of Matsuda index and AUC_{I/G} ratio.
When the analysis of the baseline data in the CC group was repeated with phenotype and gender as cofactors (two-way ANOVA), there was a trend for an effect of phenotype on Matsuda index \((p=0.085)\), with the fast phenotype displaying greater postprandial insulin sensitivity than the slow phenotype, and an effect of gender on the Matsuda*\(\text{AUC}_{iG}\) product \((p=0.038)\), with females displaying greater postprandial beta cell function than males, as was previously observed when all participants from both groups were analysed together.

When fasted beta cell function (HOMA2\(\%\B\)) was compared between visits with phenotype and gender as cofactors (two-way RM ANOVA) there were trends for visit \((p=0.053)\), visit * phenotype \((p=0.052)\), visit * gender \((p=0.071)\) and visit * phenotype * gender \((p=0.088)\). Examination of the data reveals little change in male fast, male slow and female slow phenotypes between visits, with an apparent decrease in fasted beta cell function at visit 2 for females with the fast phenotype (Figure 7.4.2.4-1).

Figure 7.4.2.4-1  HOMA2\(\%\B\) by visit, phenotype and gender, within the coffee group.
Two-way RM ANOVA revealed trends for an effect of visit, visit * phenotype, visit * gender and visit * phenotype * gender \((p<0.089)\). Error bars are SEM (fast females: \(n=3\); fast males: \(n=7\); slow females: \(n=6\); slow males: \(n=3\)).
7.4.2.5 Triacylglycerol (TAG)

*Baseline data: all participants (n=27)*

When baseline data for the whole cohort were analysed by phenotype there were no significant differences found between fast and slow phenotypes for fasted TAG (unpaired t test, p=0.250) or IAUC (Mann-Whitney U, p=0.720), nor was there any phenotype * time interaction (RM ANOVA, p=0.472). The results did not change significantly when gender was added as a cofactor. The 2 h postprandial TAG response for each phenotype is shown in Figure 7.4.2.5-1.

*Figure 7.4.2.5-1  Baseline postprandial TAG response for all participants, by phenotype. There were no significant differences between phenotypes either for fasted TAG (unpaired t test, p=0.250) or IAUC (Mann-Whitney U, p=0.720). Error bars are SEM (fast: n=13; slow: n=14).*
**Effect of phenotype in coffee group (n=19)**

There was no effect of phenotype on TAG in the CC group at baseline, either for fasted TAG (unpaired t test, p=0.478), IAUC (Mann Whitney U, p=0.905) or 2 h postprandial response (RM ANOVA, p>0.641), nor were there any differences in fasted or postprandial TAG between the two phenotypes in their response to the CC intervention (RM ANOVA, p>0.303). The 2 h postprandial TAG response at each visit split by phenotype is shown in Figure 7.4.2.5-2.

**Figure 7.4.2.5-2** Postprandial TAG response by visit and phenotype, within the coffee group.
Panel A: slow; Panel B: fast. There was no difference in the effect of coffee between phenotypes, either for fasted TAG or the 2 h postprandial response (p>0.303). Error bars are SEM (fast: n=10; slow: n=9).
When fasted TAG was compared between visits with phenotype and gender as cofactors (two-way RM ANOVA) there was an overall effect of gender ($p=0.042$), with males having higher fasted TAG than females across both visits (Figure 7.4.2.5-3).

![Figure 7.4.2.5-3](image.png)

**Figure 7.4.2.5-3** Fasted TAG by visit, gender and phenotype, within the coffee group.
There was an overall effect of gender (two-way RM ANOVA, $p=0.042$). Error bars are SEM (fast females: n=3; fast males: n=7; slow females: n=6; slow males: n=3).
An overall effect of gender (p=0.045) was also found when the time-point data were analysed by two-way RM ANOVA, with phenotype and gender cofactors, with males having an overall higher postprandial TAG response than females across both visits (Figure 7.4.2.5-4).

**Figure 7.4.2.5-4 Postprandial TAG response by visit, phenotype and gender, within the coffee group.**
Panel A: males with slow phenotype; Panel B: males with fast phenotype; Panel C: females with slow phenotype; Panel D: females with fast phenotype. There was an overall effect of gender (two-way RM ANOVA, p=0.045). Error bars are SEM (fast females: n=3; fast males: n=7; slow females: n=6; slow males: n=3).
7.4.2.6 Non-Esterified Fatty Acids (NEFA)

**Baseline data: all participants (n=27)**

When all baseline data were analysed by phenotype there were no differences found between fast and slow phenotypes for NEFA AUC (Mann Whitney U, p=0.169), nor was there any phenotype * time interaction (RM ANOVA, p=0.604), however there was a trend for a difference in fasted NEFA between the fast and slow groups (unpaired t test, p=0.062). The 2 h postprandial NEFA response for each phenotype is shown in Figure 7.4.2.6-1.

![Figure 7.4.2.6-1 Baseline postprandial NEFA response for all participants, by phenotype.](image)

There was a trend for a difference between phenotypes in fasted NEFA (unpaired t test, p = 0.062), but no difference in the 2 h AUC (Mann-Whitney U, p=0.169). Error bars are SEM (fast: n=13; slow: n=14).

When gender was added to the baseline analysis as a cofactor, the previously observed trend for a difference in fasted NEFA between phenotypes became significant (two-way ANOVA, p=0.036). When gender was added as a cofactor in the time-point analysis (two-way RM ANOVA), a time * gender effect (p=0.016) and a gender effect (p=0.028) were observed, as previously discussed in Chapter 6, along with a strong trend for an effect of phenotype (p=0.055). Post hoc analysis was employed to determine the source of these differences; one-way RM ANOVA was utilised to independently examine the effect of gender within phenotype and then to
examine the effect of phenotype within gender. This *post hoc* analysis revealed a time * gender effect (p=0.030) and a trend for an effect of gender (p=0.062) in the slow phenotype only, with no significant differences between genders in the fast phenotype and no significant effects of phenotype for either males or females. This suggests that most of the gender differences occurred within the slow phenotype. Examination of the baseline response (Figure 7.4.2.6-2) reveals little or no NEFA suppression in the slow male subgroup in comparison with the slow females.

![Figure 7.4.2.6-2 Baseline postprandial NEFA response for all participants, by phenotype and gender.](image)

Two-way RM ANOVA revealed an effect of gender (p=0.028), a time * gender effect (p=0.016) and a trend for an effect of phenotype (p=0.055). Error bars are SEM (fast females: n=4; fast males: n=9; slow females: n=9; slow males: n=5).

**Effect of phenotype in coffee group (n=19)**

Baseline NEFA values were different between the phenotypes in the CC group, with the fast phenotype exhibiting a lower fasted NEFA than the slow phenotype (unpaired t test, p=0.005) and a lower total AUC (Mann-Whitney U, p=0.010). There were also significant differences between fast and slow phenotypes at 15 and 30 min time-points (Mann-Whitney U, p<0.011).

When gender was added as a cofactor in the baseline analysis (two-way ANOVA), there were significant effects of gender (p=0.006) and of phenotype (p<0.001) on fasted NEFA, with females having lower fasted NEFA than males and the slow phenotype having a higher fasted NEFA than the fast phenotype.
When gender was added as cofactor in the baseline time-point analysis in the CC group (two-way RM ANOVA), significant effects of phenotype (p=0.001) and gender (p=0.006) were observed, along with trends for phenotype * gender (p=0.069), time * gender (p=0.055) and time * gender * phenotype (p=0.052). Post hoc analysis was employed to determine the source of these differences; one-way RM ANOVA was utilised to independently examine the effect of gender within phenotype and then to examine the effect of phenotype within gender. This post hoc analysis revealed a time * gender effect (p=0.07) and an effect of gender (p=0.024) in the slow phenotype only and an effect of phenotype in males (p=0.009) with a trend for an effect of phenotype in females (p=0.086). This suggests that most of the gender differences occur within the slow phenotype and that most of the effect of phenotype occurs within males. Examination of the baseline response (Figure 7.4.2.6-3) reveals little or no NEFA suppression in the slow male subgroup, with all other subgroups displaying a degree of NEFA suppression with some smaller differences between these subgroups.

![Figure 7.4.2.6-3 Baseline postprandial NEFA response by phenotype and gender, within the coffee group. Two-way RM ANOVA revealed a significant effect of phenotype (p=0.001) and gender (p=0.006). Error bars are SEM (fast females: n=3; fast males: n=7; slow females: n=6; slow males: n=3).]
The difference between phenotypes was non-significant at visit 2 for both fasted NEFA and AUC values (Mann-Whitney U, p>0.112), however there was a difference between phenotypes at 60 min (Mann-Whitney U, p=0.028) and a trend for a difference at 120 min (p=0.053) for visit 2. One-way RM ANOVA on the time-point data revealed a visit * time * phenotype interaction (p=0.044) and a trend for an effect of phenotype (p=0.056). The 2 h postprandial response by visit for each phenotype in the CC group is shown in Figure 7.4.2.6-4.

![Figure 7.4.2.6-4 Postprandial NEFA response by phenotype and visit, within the coffee group. There was a difference in fasted NEFA between phenotypes at visit 1 only (Mann-Whitney U, p=0.006). One-way RM ANOVA on time-point data found a visit * time * phenotype effect (p=0.044). Error bars are SEM (fast: n=10; slow: n=9).]
When the change in fasted NEFA between visits was analysed by two-way RM ANOVA with phenotype and gender as cofactors, there was a significant effect of phenotype (p=0.005) and gender (p=0.022), along with phenotype * gender (p=0.027) and visit * phenotype * gender (p=0.036) interactions. The change in fasted NEFA response between visits, split by phenotype and gender is shown in Figure 7.4.2.6-5.

![Figure 7.4.2.6-5 Fasted NEFA by visit, phenotype and gender, within the coffee group.](image)

*Two-way RM ANOVA found a significant effect of phenotype (p=0.005) and gender (p=0.022), along with phenotype * gender (p=0.027) and visit * phenotype * gender (p=0.036) interactions. Error bars are SEM (fast females: n=3; fast males: n=7; slow females: n=6; slow males: n=3).*

Two-way RM ANOVA on the time-point data with phenotype and gender as cofactors revealed significant effects of phenotype (p=0.003) and gender (p=0.010), along with gender * phenotype (p=0.014), visit * time * phenotype (p=0.026), time * gender (p=0.016), and visit * time * gender * phenotype (p=0.020) interactions and a trend for a time * phenotype interaction (p=0.084). *Post hoc* analysis was employed to determine the source of these differences; one-way RM ANOVA was utilised to independently examine the effect of gender within phenotype and also to examine the effect of phenotype within gender. This *post hoc* analysis revealed a slight trend for a visit * gender effect (p=0.093) and visit * time * gender (p=0.089) for the fast phenotype (panels B and D, Figure 7.4.2.6-6), and a time * gender (p=0.049) and gender effect (p=0.025) in the slow phenotype (panels A and C). The analysis also found a visit * phenotype effect (p=0.020) and a visit * time * phenotype effect (p=0.019) for females only (panels C and D). There was no visit * phenotype effect (p=0.322) in males (panels A and B) but there was an overall effect of phenotype (p=0.007).
This suggests that slow males and females had a different response to each other, but no difference in their response to the intervention, whereas there was a trend for a difference between the fast males and females between visits. Furthermore, although the fast and slow males had a different postprandial response to each other, there was no difference between them in their response to the intervention, whereas the fast and slow female phenotypes responded differently to the intervention. Examination of the change between visits for each gender/phenotype subgroup (Figure 7.4.2.6-6) reveals the differences to be most apparent between fast and slow females over the first 60 min, mainly as a result of differences in fasted NEFA at visit 2, with the fast females having higher fasted NEFA at the second visit.

![Figure 7.4.2.6-6 Postprandial NEFA response by visit, phenotype and gender, within the coffee group.](image)

Two-way RM ANOVA revealed significant effects of phenotype (p=0.003) and gender (p=0.010), along with gender * phenotype (p=0.014), visit * time * phenotype (p=0.026), time * gender (p=0.016), and visit * time * gender * phenotype (p=0.020) interactions. Error bars are SEM (fast females: n=3; fast males: n=7; slow females: n=6; slow males: n=3).
7.4.2.7 Total cholesterol

**Baseline data: all participants (n=27)**

There were no differences between fast and slow phenotypes for baseline total cholesterol (unpaired t test, \( p=0.254 \)). This did not change significantly when gender was added as a cofactor.

**Effect of phenotype in coffee group (n=19)**

When baseline data for the CC group were analysed, there were no differences between fast and slow phenotypes for total cholesterol (unpaired t test, \( p=0.298 \)). This did not change significantly when gender was added as a cofactor.

Total cholesterol in the CC group was compared between visits by one-way RM ANOVA with phenotype as cofactor. No effect of visit, phenotype or visit * phenotype effect was found (\( p>0.123 \)), nor was there a difference between phenotypes at baseline (unpaired t test, \( p=0.298 \)), however there was a trend for a difference between phenotypes at visit 2 (unpaired t test, \( p=0.080 \)) with the fast phenotype exhibiting lower total cholesterol. There were no significant changes when gender was added as a cofactor in the analysis. Fasted cholesterol by visit for each phenotype in the CC group is shown in Figure 7.4.2.7-1.

![Figure 7.4.2.7-1](Image)

**Figure 7.4.2.7-1 Mean total cholesterol by visit and phenotype, within the coffee group.**

There was no difference between phenotypes at baseline (unpaired t test, \( p=0.298 \)), however there was a trend for a difference at visit 2 (unpaired t test, \( p=0.080 \)). Error bars are SEM (fast: \( n=10 \); slow: \( n=9 \)).
Chapter 7.

7.4.2.8 HDL cholesterol

**Baseline data: all participants (n=27)**

There were no differences between fast and slow phenotypes in both groups for baseline HDL cholesterol (unpaired t-test, \( p=0.623 \)). Adding gender as cofactor did not significantly change this result.

**Effect of phenotype in coffee group (n=19)**

One-way RM ANOVA was used to compare changes in HDL cholesterol between visits, by phenotype, in the CC group. No effect of visit, phenotype or visit * phenotype effect was found (\( p>0.232 \)) nor was there any difference between phenotypes at baseline (unpaired t test, \( p=0.362 \)) or visit 2 (unpaired t test, \( p=0.261 \)). Adding gender as cofactor did not significantly change these results. HDL cholesterol by visit for each phenotype in the CC group is shown in Figure 7.4.2.8-1.

**Figure 7.4.2.8-1** Mean HDL cholesterol by visit and phenotype, within the coffee group.

There was no difference between phenotypes at baseline (unpaired t test, \( p=0.362 \)), nor were there any differences in change between visits between phenotypes (RM ANOVA, \( p>0.232 \)). Error bars are SEM (fast: \( n=10 \); slow: \( n=9 \)).
7.4.2.9 Inflammatory markers, IL-6 and TNF-α

Baseline data: all participants (n=27)

There were no differences between fast and slow phenotypes for all participants at baseline for IL-6 (Mann Whitney U, p=0.583) or TNF-α (unpaired t test, p=0.987). These results did not change when gender was added as cofactor.

Effect of phenotype on IL-6 and TNF-α in the coffee group (n=19)

When baseline values in the CC group were compared, there was no difference between phenotypes for TNF-α (unpaired t test, p=0.487), however there was a slight trend for a difference in IL-6 (Mann Whitney U, p=0.095), with the slow phenotype having higher IL-6 (Figure 7.4.2.9-1, panel A). This trend disappeared when gender was added as cofactor (p=0.100).

TNF-α was compared between visits by RM ANOVA with phenotype as cofactor. No effect of visit, phenotype or visit * phenotype effect was found (p>0.483). There was no difference between groups in the change between visits for IL-6 (Mann Whitney U, p=0.211). Adding gender as cofactor did not significantly affect any results.

Mean IL-6 and TNF-α by visit for each phenotype in the CC group is shown in Figure 7.4.2.9-1.
Figure 7.4.2.9-1  Mean IL-6 and TNF-α by visit and phenotype, within the coffee group. Panel A: IL-6; Panel B: TNF-α. There were no differences between phenotypes, either at baseline or in their response to the intervention (p>0.094). Error bars are SEM (fast: n=10; slow: n=9).
7.4.3 Summary of results

There were differences between the two phenotypes at baseline for several measures, both for the whole cohort as a group, and for those within the CC group; these are summarised in Table 7.4.3-1. When participant characteristics were examined, there was a trend for those with the slow phenotype to be older and to have lower SBP when all participants were analysed, but this trend disappeared when just the CC group were analysed. Males with the slow phenotype had a higher amount of body fat than those with the fast phenotype. Those participants with the slow phenotype had higher fasted and postprandial glucose at baseline and higher fasted and postprandial NEFA than those with the fast phenotype, although many of these differences only became apparent or reached significance after gender was included as a cofactor in the analysis. There was also a trend for higher Matsuda index in those with the fast phenotype after gender was added into the analysis.

### Table 7.4.3-1 Summary of baseline differences between fast and slow phenotypes.

<table>
<thead>
<tr>
<th>Measure</th>
<th>All participants Fast (n=13)</th>
<th>Slow (n=14) Other results</th>
<th>Coffee group Fast (n=10)</th>
<th>Slow (n=9) Other results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>↗</td>
<td>trend for SM &gt; FM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP</td>
<td>↗</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>SM &gt; FM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>trend for SM &gt; FM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight</td>
<td>trend for SM &gt; FM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasted glucose</td>
<td>↑ +G</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Postprandial glucose</td>
<td>↑ +G</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Postprandial insulin</td>
<td>S &gt; F at 90 min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Matsuda index</td>
<td>↗ +G</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasted NEFA</td>
<td>↑ +G</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Postprandial NEFA</td>
<td>↗ +G</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>↗ -G</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

↗: A trend for a higher result was observed in this phenotype (p<0.100); ↑: A statistically higher result was observed in this phenotype (p<0.050); +G: result was only observed after gender added as cofactor; -G: result disappeared after gender added as cofactor; BMI: body mass index; F: fast phenotype; FM: fast males; G: gender; NEFA: non-esterified fatty acids; P: phenotype; S: slow phenotype; SBP: systolic blood pressure; SM: slow males; T: time; V: visit.
The two phenotypes reacted differently to the CC intervention, as summarised in Table 7.4.3-2. Participants with the fast phenotype saw a worsening in their glucose response, and a reduction in HOMA2_%B, but greater NEFA suppression after the intervention, whereas those with the slow phenotype had an improvement in glucose response, but exhibited less NEFA suppression at visit 2. Both phenotypes showed a delayed peak in insulin response at the second visit, with no difference between fast and slow. Interactions between gender and phenotype were also observed for HOMA2_%B and postprandial NEFA.

Table 7.4.3-2  Summary of changes between visits for fast and slow phenotypes in the coffee group.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Within-group change</th>
<th>Between-group change</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postprandial glucose</td>
<td>✓</td>
<td>V*P; F:↑ at visit 2; S: ↓ at visit 2</td>
<td></td>
</tr>
<tr>
<td>Postprandial insulin</td>
<td>✓</td>
<td>delayed insulin peak at visit 2 in both phenotypes</td>
<td></td>
</tr>
<tr>
<td>Insulin sensitivity</td>
<td>✓</td>
<td>HOMA-2 %B: trends for V<em>P, V</em>G and V<em>P</em>G</td>
<td></td>
</tr>
<tr>
<td>Fasted NEFA</td>
<td>✓</td>
<td>P<em>G; V</em>P*G</td>
<td></td>
</tr>
<tr>
<td>Postprandial NEFA</td>
<td>✓</td>
<td>V<em>T</em>P; S:↑ at visit 2; F: ↓ at visit 2; V<em>T</em>P*G</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>✓</td>
<td>trend for F &lt; S at visit 2</td>
<td></td>
</tr>
</tbody>
</table>

F: fast phenotype; G: gender; NEFA: non-esterified fatty acids; P: phenotype; S: slow phenotype; T: time; V: visit

7.5 Discussion

7.5.1 Baseline differences between phenotypes

The differences between genotypes observed at baseline are somewhat surprising given that the increased enzyme activity associated with the fast metaboliser phenotype requires induction by, for example, regular caffeine use or smoking, and the participants reported to be non-smokers and non-coffee drinkers. They were, however, not caffeine-naïve, having a median weekly caffeine intake of 80 mg (range: 0 - 410 mg). It has been suggested that “heavy” coffee consumption of at least 3 cups/day is required to induce the fast phenotype. Three cups of coffee per day equates to a minimum weekly caffeine intake of over 1000 mg, so it seems unlikely that any of the participants had a sufficiently high caffeine intake to have had the increased enzyme activity characteristic of the fast phenotype. The data imply that there is some as yet uncharacterised effect of this genotype, independent from its effects on caffeine metabolism, which has resulted in the baseline differences. Giving
weight to this argument is a recent study which examined associations between several SNPs and T2DM risk and found an association between carriers of the rs762551 C allele (conferring a slow phenotype) and increased T2DM risk irrespective of actual coffee consumption (159). They also found that being a carrier of the slow allele was not predictive of coffee intake, suggesting that it is not simply lower coffee consumption causing the increased risk in this group. Interestingly, they also observed a coffee consumption * gender interaction effect on T2DM risk, with their data suggesting a protective effect of high coffee consumption in women, but not in men. However, the protective effect in women was not statistically significant, leading the authors to conclude that they had insufficient power in their sample size.

An equally viable hypothesis is that there was some other, unaccounted for, factor common to participants in each phenotype group that caused the differences observed in the current study, with phenotype having no effect.

7.5.2 Effects on glucose and insulin metabolism

There was no difference between the two phenotypes at baseline for either fasted or postprandial glucose until gender was added into the analysis as a cofactor, although there was a difference at the 90 min time-point in the initial analysis, which is perhaps indicative of an overall effect. Given the known differences between men and women in glucose metabolism, and the differences observed between genders in both fasted glucose and postprandial glucose response, as discussed in Chapter 6, it is quite possible that gender was confounding the results until it was accounted for in the analysis. This is particularly likely when one considers the uneven, albeit non-significant, distribution of genders observed between phenotypes (4 male: 9 female with the fast phenotype; 9 female: 5 male with the slow phenotype). Once gender was included in the analysis, a significant difference between phenotypes was observed at baseline for both fasted glucose and postprandial glycaemic response, with the slow phenotype displaying higher fasted and postprandial glucose. Females were also observed to have lower fasted and postprandial glucose than men, as previously discussed in Chapter 6.

Whilst differences were anticipated between phenotypes in response to the CC intervention, the differences observed at baseline were unexpected. The fast phenotype displaying lowerfasted glucose and a lower postprandial glucose response is however consistent with the epidemiology, previously discussed, which suggests that coffee drinking is associated with greater risk of IFG only in those with the slow phenotype. When the 2 h postprandial glucose response for each gender/phenotype
combination is examined (Figure 7.4.2.2-2) the response in the fast female subgroup appears markedly different from the other subgroups, with these participants displaying very little postprandial increase in plasma glucose and indeed, post hoc analysis, examining the genders separately, revealed a difference between fast and slow phenotypes for women only.

A difference was observed between the two phenotypes in their response to the CC intervention, with the visit * phenotype interaction indicating an increase in the postprandial glucose response in those with the fast phenotype and a reduction in those with the slow phenotype, however post hoc analysis suggests an effect in the fast phenotype only, with those in the fast female subgroup being most affected by the intervention. When the number of participants in each subgroup is considered, it is likely that the analysis lacked sufficient power to detect smaller differences in some subgroups. This apparent worsening of the glucose response in those with the fast phenotype is in contrast to the epidemiology which suggests that carriers of the slow allele are at greater risk of developing T2DM and IFG. However it should be considered that most of the observed effect was in the fast female subgroup which had better glycaemic control at baseline. This may simply be a case of regression to the mean, as female fast and slow phenotypes had a very similar postprandial glycaemic response at the second visit.

There were no significant differences between fast and slow phenotypes at baseline for either fasted or postprandial insulin, although there was a slight trend for the slow phenotype to have higher insulin at the 90 min time-point, which may be indicative of a difference. There was also no difference in the effect of the CC intervention on either of these measures.

There was a trend for greater postprandial insulin sensitivity, as measured by Matsuda index, in those with the fast phenotype at baseline. There was also an effect of gender on postprandial beta cell function, with women having a higher Matsuda * AUCIG product at baseline and women with the fast phenotype exhibiting a decrease in postprandial beta cell function at visit 2. These differences, whilst contrary to the epidemiology previously discussed, are consistent with the observed differences in glucose metabolism between genders and phenotypes.
7.5.3 Effects on lipid metabolism

There were no differences at baseline between phenotypes for total or HDL cholesterol, however there was a trend for the fast phenotype to have lower total cholesterol than the slow phenotype at visit 2. This suggests that having the fast phenotype may confer some benefit in terms of lipid profile. It should be noted, however, that both phenotypes had total cholesterol levels within the healthy range.

There were also no significant differences between phenotypes for fasted or postprandial TAG at baseline or in their response to the CC intervention. However, as discussed in Chapter 6, postprandial TAG was only measured for 2 h; if measurements had continued for several hours, beyond the postprandial peak, it is possible differences would be observed. Furthermore, little increase in TAG was observed during that 2 h period, if the meal had provided a greater fat challenge a larger increase might have been observed.

There were differences between phenotypes at baseline for both fasted and postprandial NEFA when gender was included in the model as a cofactor, with the slow phenotype having higher fasted NEFA and, consequently, higher overall postprandial NEFA, despite similar levels of NEFA suppression. Both slow and fast males displayed little postprandial NEFA suppression in comparison with the females, as previously discussed in Chapter 6.

When baseline data in the CC group were analysed, a phenotype * gender effect was observed along with effects of phenotype and gender, with females having lower overall NEFA and greater NEFA suppression than males. This difference in response is consistent with the baseline differences observed between phenotypes for insulin sensitivity, where the fast phenotype was observed to have greater postprandial insulin sensitivity than the slow phenotype and females displayed greater postprandial beta cell function than males. Closer examination revealed the slow male subgroup to exhibit very little postprandial NEFA suppression in comparison to the other subgroups. It should be noted however, that there were only three participants in this subgroup, too small a number to draw any firm conclusions. Also, this subgroup were older and had a higher mean body fat percentage than the fast male subgroup. The known effects of age and body fat on insulin resistance may be contributing to the observed difference and may be confounding the results.

The two phenotypes responded differently to the CC intervention, with the slow phenotype displaying less postprandial NEFA suppression at the second visit and the fast phenotype displaying greater postprandial suppression, despite an increase in
fasted NEFA. As the fast phenotype had a lower overall NEFA response than the slow phenotype at baseline, this has resulted in a greater difference between the two phenotypes after the intervention and is indicative of a potential detrimental effect of CC on those with a slow phenotype.

When gender was included in the analysis and the various phenotype/gender subgroups examined, there was an apparent difference in the way the genders and phenotypes reacted to the intervention, with the main difference appearing to be between the fast and slow females. The fast females exhibited an increase in fasted NEFA, consistent with the observed decrease in fasted beta cell function previously discussed. Despite this increase, there was no difference between visits from the 60 min time-point onwards. Again, care should be taken when drawing conclusions from the subgroup analysis due to the small numbers involved.

7.5.4 Effects on inflammatory markers

There were no apparent effects of phenotype on the inflammatory markers, IL-6 and TNF-α, either at baseline or in response to the CC intervention. As discussed in Chapter 6, the participants displayed large inter-individual variation in these markers, which could be masking small differences between the phenotypes.

7.5.5 Potential confounders

A number of SNPs, other than rs762551, have been reported to be associated with glucose metabolism. A recent study found associations between 51 individual SNPs and various measures of CHO metabolism, including five SNPs associated with fasted glucose and six associated with fasted insulin \(^{(260)}\), however they did not measure rs762551, or indeed any others on the CYP1A2 gene, so direct comparisons cannot be made. As none of these other SNPs were measured in the current study they are potential confounders to the results. Similarly, several other SNPs on the CYP1A2 gene, for example rs2069514 and rs12720461, are associated with decreased enzyme activity and are therefore also potential confounders. The rs2069514 SNP in particular may be a confounder; a cross-sectional study in Japanese men found rs2069514 modified the association between coffee drinking and prevalence of T2DM, although they found no effect of either rs2069514 or rs762551 on the association between coffee drinking and IFG/IGT \(^{(261)}\). The A allele of the rs2069514 SNP, which confers
the slow metaboliser phenotype, is uncommon in Caucasians (8%), but is relatively common in Asians (28%); as 41% of the study group were Asian it is likely that some participants were carriers of this allele.

7.5.6 Weaknesses

Whilst the fast and slow phenotype groups were not statistically different in terms of gender, ethnicity, age and baseline anthropometrics, the gender split in particular was uneven and there were several baseline differences between males with the fast and slow phenotypes. It was not possible to recruit by genotype for financial reasons, however this would have ensured a more even balance of genders, age, and adiposity across groups. Also, although some potentially interesting gender/phenotype interactions were observed, the study was underpowered for this post hoc analysis.

7.6 General discussion/conclusion

At baseline, the slow phenotype was observed to have higher fasted and postprandial glucose, higher fasted NEFA and lower postprandial insulin sensitivity. Taken together, this suggests a less healthy overall physiological state in those with the slow phenotype, however it is not known whether this difference is a result of random chance, or whether it has been caused by this genotype, particularly as in theory the fast phenotype should not have been “activated” at this point. It is however consistent with the epidemiology observing an association between carriers of the slow C allele and T2DM risk irrespective of coffee consumption, as discussed previously.

Following twelve weeks of CC consumption, an increase was observed in the postprandial glucose response in those with the fast phenotype. This was unexpected and suggests a potentially detrimental effect of habitual CC drinking on the glucose response in this phenotype, contrary to the epidemiology, although it should be noted that this increased glucose response was not abnormally high and glucose levels at the end of the test were no different from those measured at the first visit. In contrast, the coffee intervention resulted in greater NEFA suppression in the fast phenotype and less NEFA suppression in the slow phenotype. This apparent worsening in the postprandial NEFA response in the slow phenotype is particularly interesting when one considers that they exhibited poorer levels of NEFA suppression at baseline than the fast phenotype and is indicative of a potentially detrimental effect of CC in this
phenotype. It should however be noted that there were differences in adiposity between the subgroups, which might be confounding the results as higher amounts of adipose tissue are associated with greater insulin resistance and consequently higher circulating NEFA.

The differences at the gender/phenotype subgroup level are potentially very interesting, particularly the apparently greater differences observed between the two female subgroups in their response to the intervention, however it should be remembered that numbers in these subgroups were very small. With such low numbers no conclusions can be drawn from these results, however it does serve to highlight this as a potentially interesting area for further study.

In conclusion, this is the first study to our knowledge to compare the effects of habitual CC drinking on fast and slow caffeine metabolisers, as determined by the rs762551 SNP. The observed baseline differences suggest that coffee-naïve individuals who are carriers of the slow C allele may exhibit a worse glucose and lipid profile than those homozygous for the fast A allele. Supporting the hypothesis that the two phenotypes would respond differently to the intervention, a potentially detrimental effect of CC was observed on glucose response in the fast phenotype and on lipid response in the slow phenotype. These differences between phenotypes make it inadvisable at this stage to recommend either coffee drinking or abstention to either group, particularly when the apparent gender differences are considered. More work is clearly required in order to investigate these differences further.
Chapter 8. General discussion

There is strong epidemiological evidence demonstrating an association between coffee drinking and reduced risk of T2DM, however epidemiology cannot demonstrate a causal relationship and results from interventional studies have been somewhat contradictory, with many acute studies demonstrating a detrimental effect of CC consumption on the postprandial glycaemic response.

Studies examining the acute effects of coffee drinking have typically used a single large dose of coffee. Little was known about the acute effects of a more normal dose, such as a single serving of instant coffee, as is commonly consumed in the UK. Moreover, previous studies have examined the effects of coffee taken first thing in the morning when people are at their most insulin sensitive. It was not known whether people would respond differently to coffee consumed later in the day when glucose tolerance declines. A possible confounder to interpretation of previous studies is the lack of data on the composition of the individual coffees. Caffeine is known to increase the postprandial glycaemic response, whereas CGAs have been demonstrated to lower it. It is possible that different proportions of these coffee components may explain the varying results reported by previous studies, however the majority have failed to report their coffee composition.

There have been few previous studies examining the longer-term effects of coffee drinking, with the majority of these having examined the effects in regular coffee drinkers. If there are beneficial effects of coffee drinking in the longer term then one would expect habitual coffee drinkers to have already adapted to these changes and so they are perhaps not the ideal candidates for a longer-term intervention. Another possible confounder to previous work is a particular SNP in the CYP1A2 gene which determines whether one is a fast or slow caffeine metaboliser. The slow phenotype of this SNP has been associated with increased risk of T2DM and with IFG in coffee drinkers; it is possible that it might also affect how people respond to coffee interventions, however to our knowledge, this has not previously been examined.

The aims for this thesis were to address some of these gaps in the existing knowledgebase. A systematic review (Chapter 3) was carried out on all studies to date that had examined the acute effects of coffee on postprandial glucose metabolism, to investigate whether there were any patterns explaining the apparent inconsistency in results between studies. The first experimental study (Chapter 4) had three aims: firstly to investigate the acute effects of a single “normal” serving of instant CC on the
postprandial glycaemic response; secondly, to establish whether there was any dose-
response relationship; and thirdly, to investigate whether there was any dose-response
effect of increasing the non-caffeine components of coffee when taken along with the
amount of caffeine typically found in a single serving of instant CC. The second
experimental study (Chapter 5) investigated the effects of a single serving of DC on
the postprandial glucose and insulin response when taken at different times of day. It
also examined whether there was any carry-over effect of morning DC on the lunchtime
postprandial response and whether there were any effects of repeated dosing. The
final experimental study (Chapters 6 and 7) was a longer-term intervention
investigating the effects of regular CC drinking in coffee-naïve individuals, with further
analysis examining whether there was any difference in the response to the CC
intervention between fast and slow caffeine metabolisers.

8.1 Overview of main findings
Twenty studies were eligible for inclusion in the systematic review (Chapter 3). All
investigated the acute effects of CC and/or DC on the postprandial glycaemic
response. The methodologies employed by these studies were varied. Coffee doses
ranged from a rounded teaspoon of instant coffee up to the equivalent of six teaspoons
of instant coffee, taken as a single dose, with the majority utilising relatively high doses.
Types of coffee varied and included instant, filter and espresso coffees; the timing of
the coffee dose ranged from one hour before the test meal to concurrent dosing. Seven
studies did not report the caffeine content of their coffees and only two studies reported
total CQA or CGA content. The amount of CHO in the tests ranged from 24 – 75 g and
was provided either in a mixed meal or as a glucose drink. A mix of capillary and
venous blood sampling techniques were employed. The number of participants ranged
from 8 to 33, with the majority recruiting young healthy adults. Despite the
heterogeneity of the study designs, the majority of studies utilising CC reported an
increased postprandial glucose response following CC consumption, although most of
these only observed an increase at isolated time-points, with only five studies
observing an effect on the overall glucose response. There was less evidence for a
detrimental effect of CC on the postprandial insulin response. Furthermore the majority
of studies observed no effect of DC on the acute postprandial glucose and insulin
responses. A detailed examination of the differences in methodology provided no
obvious explanation for the lack of uniformity in the reported results. However, as most
of the studies did not report a detailed analysis of their coffee, and with several failing
to report the caffeine content, it was not possible to determine whether varying proportions of the different coffee components had determined the observed results.

The first interventional study carried out for this thesis (Chapter 4) was split into two parts. The first part examined the effects of escalating doses of instant DC, with caffeine added in proportion to the DC dose, on the postprandial glucose and insulin response. The doses given to the participants were the equivalent of one, two and four servings of regular instant CC. Similarly to the majority of studies in the systematic review, an increase in the postprandial glucose response was observed following CC consumption, for both the one and two serving equivalents, with no effect on insulin. Interestingly no significant effect was observed following the highest dose, equivalent to four servings of CC, nor was there any dose-response effect. The second part of the study examined the effects of the same three doses of DC as used in Part A, but this time with a constant amount of caffeine added to each drink, including control. The amount of added caffeine was set to the lowest caffeine dose from Part A of the study. No differences were observed in the postprandial glucose and insulin response between any of the test drinks and the control in this part of the study.

The second study (Chapter 5), investigated the acute effects of DC consumed at different times of day on the postprandial glucose and insulin response. Similarly to the studies in the systematic review, there were no observed effects of a single serving of DC taken in the morning on either measure. Nor was there any carry-over effect of DC consumed in the morning on the postprandial response to a later meal. There were however some interesting trends for an effect of DC consumed at lunchtime, with a single serving of DC at lunchtime resulting in a reduced glucose peak. Furthermore, a reduction in the lunchtime postprandial insulin response was observed when DC was consumed at breakfast and lunch.

The final study was a longer-term parallel-arm intervention, examining the effects of twelve weeks of CC consumption on glucose and lipid metabolism in coffee-naïve individuals. In the primary analysis (Chapter 6), no effect of longer-term CC drinking on fasted or postprandial glucose response was observed, nor were there any observed effects on fasted and postprandial lipid metabolism. However, an effect on the postprandial insulin response was observed, with the participants in the CC group displaying a delayed insulin peak following the intervention. Differences between genders at baseline were observed, with females having lower fasted and postprandial glucose and TAG and greater postprandial NEFA suppression, however there were no differences between males and females in their responses to the intervention.
In the secondary analysis (Chapter 7), the baseline data for all participants was analysed to determine whether there were any innate differences between those with the fast and slow caffeine-metaboliser phenotypes. This revealed some interesting differences, with the slow phenotype exhibiting a less healthy profile, with higher fasted and postprandial glucose and NEFA. Analysis of the CC group was then performed to determine whether there were any differences between the two phenotypes in their response to the intervention. This revealed further differences, with participants with the fast phenotype displaying an increased postprandial glucose response and a reduction in HOMA2_%B but greater NEFA suppression after the intervention. In contrast, those with the slow phenotype displayed a reduction in postprandial glucose and less postprandial NEFA suppression.

Finally, subgroup analysis was performed to determine whether there were any interactions between male and female, fast and slow phenotypes. It should however be noted that numbers in these subgroups were very low and these results are reported solely for purposes of hypothesis generation. This subgroup analysis suggests that the baseline difference in the postprandial glucose response between phenotypes was primarily due to a difference between fast and slow females, with little difference observed in the males. Furthermore when the response to the intervention was examined, the effect of phenotype on the postprandial glucose response was most apparent in women, with the greatest difference observed in the fast female subgroup, who displayed a noticeable increase in glucose response. Subgroup analysis of the baseline NEFA data revealed most of the gender/phenotype differences in NEFA suppression to be found in the slow phenotype, with slow males exhibiting minimal postprandial NEFA suppression in comparison with other subgroups. Interestingly, although the fast phenotype displayed increased NEFA suppression overall, when the subgroups were analysed the biggest effect was observed in the fast females who displayed an increased NEFA AUC following the intervention, as a result of an increase in fasted NEFA.
8.2 Study limitations

Limitations of the individual studies were discussed in detail in their respective chapters; only the main limitations are summarised here.

The participants in these studies were mainly young, healthy individuals. These results cannot be extrapolated to other populations, such as those with IFG/T2DM who may react differently to both acute and longer-term coffee ingestion.

The acute studies, whilst using the recommended number of participants for GI testing, were likely underpowered to detect a dose-response effect. Similarly the longer-term study was underpowered to detect the unexpected differences observed between genders and phenotypes, although these were secondary endpoints.

The CGA content in the coffees was relatively low, both in the DC used in the two acute studies and in the CC used in the longer-term intervention. It is possible that different effects may have been observed if different coffees had been used, however this is coffee on sale to the UK public and therefore is representative of typical consumption.

The coffee doses were not tailored to match the participants’ body sizes. This may have confounded the results, however this approach was taken to reflect normal consumption patterns rather than artificial laboratory conditions.

Retrospective genotyping and analysis was performed on the participants in the longer-term intervention, due to financial constraints. Recruitment by genotype would however have ensured a better balance between genders and genotypes which would have helped the interpretation of the subgroup analysis.

8.3 Comparison of acute and longer-term studies

The first two studies reported in this thesis examined the acute effects of coffee, both caffeinated (Chapter 4) and decaffeinated (Chapter 5) on the 2 h postprandial glucose and insulin response. Consistent with the majority of studies reported in the systematic review (Chapter 3), these acute studies reported an increased postprandial glycaemic response following a single dose of CC, with no effect of a single dose of DC. These results, along with studies examining the effects of caffeine in isolation, support the hypothesis that it is the caffeine component of coffee that produces the temporary worsening in glycaemic response following coffee consumption.
The same brand, batch and dose of DC was used in both acute studies, with the only difference in treatment being the addition of caffeine in the CC study. However, the CC study provided 50 g CHO in the form of glucose added to the coffee, whereas the DC study provided 75 g CHO as a mixed meal. Whilst the higher amount of CHO in the DC study would likely produce a larger overall glycaemic response, the rate of absorption would be attenuated by the presence of protein and fat in the mixed meal \(^\text{[262]}\). This difference in CHO and macronutrient composition means that any comparison of the postprandial response between studies should be interpreted with caution.

Furthermore, the participants recruited to each study were from different sample populations. The participants in the CC study were overweight males (mean BMI: 27.8 kgm\(^{-2}\)), whereas the participants in the DC study were normal-weight females (mean BMI 21.6 kgm\(^{-2}\)). As discussed in previous chapters, overweight people tend to be more insulin resistant than normal weight and males tend to have higher fasted glucose, but lower 2 h glucose following an oral glucose challenge. In support of the epidemiology, although not tested statistically, the males in the CC study appear to have a higher fasted glucose (mean: 5.2 mmol/L) than the females in the DC study (mean: 4.8 mmol/L). Furthermore plasma glucose concentrations for the males in the CC study had returned to baseline after 2 h, in contrast to the females in the DC study.

When the postprandial glycaemic responses to the control drinks are compared, the responses from the two study populations appear different. Whilst not tested statistically, the mean peak glucose for the CC study (8.3 mmol/L) appears to be higher than that observed for the DC study (7.5 mmol/L), despite similar peak insulin concentrations (CC: 193 pmol/L; DC: 183 pmol/L). This suggests that the overweight men in the CC study may be more insulin resistant, particularly when one considers the smaller amount of CHO utilised in this study. The higher peak may also be a consequence of the liquid glucose being absorbed faster than the mixed meal.

However, as the IAUCs were similar between treatments (CC: 172 mmol/L.120 min; DC: mean 160 mmol/L.120 min), despite the larger CHO load in the DC study, it appears likely that the overweight men were more insulin resistant.

The longer-term study (Chapters 6 and 7) did not give an acute dose of CC on either study day as its aim was to examine the chronic effects of coffee drinking. It would be interesting to compare the baseline postprandial response from this study group with those from the control visits of the two acute studies, to examine whether there were any differences in response between coffee-naïve individuals (longer-term study) and regular coffee drinkers (acute studies). However, in addition to differences in sample
populations, as discussed in relation to the acute studies, there were differences between the acute and longer-term studies both in blood sampling and laboratory analysis methods, rendering this comparison inappropriate.

The two acute studies utilised the capillary sampling methodology, whereas the longer-term study employed venous sampling via cannula. Capillary sampling is widely regarded as the gold standard method for GI testing as capillary blood is primarily arterial blood and therefore provides a more accurate representation of glucose absorption. Conversely venous blood glucose concentrations tend to be lower as a result of glucose disposal into muscle and other tissues before reaching the veins. The results from the longer-term study appear to support this as mean baseline fasted and peak glucose concentrations (4.6 and 6.3 mmol/L respectively) were lower than those observed in both acute studies, despite this group exhibiting a higher mean BMI (23.2 kgm\(^{-2}\)) than that observed in the DC study. However, these differences are relatively small, particularly for fasted glucose concentrations, and may be confounded by a difference in glucose analysis methodology. Samples from both acute studies were analysed on the YSI 2300 Stat Plus analyser, whereas samples from the longer-term study were analysed on the ILab 650. Whilst both analysers utilise the glucose oxidase reaction, there are nevertheless likely to be small differences in the absolute values reported.

As discussed previously, blood from capillary samples tends to have lower insulin concentrations than venous samples. This is apparent when the insulin responses for each study are examined, with mean insulin concentrations for the longer-term study (fasted: 64 pmol/L; peak: 660 pmol/L) almost threefold that of the acute studies (fasted: 23 and 27 pmol/L; peak: 183 and 193 pmol/L). As with the glucose analysis, different methodologies were employed for the insulin analysis. A comparison of 11 different human insulin assays reported that RIA returned higher insulin concentrations than ELISA and further that serum samples resulted in higher insulin concentrations than plasma \(^{263}\). As the acute studies analysed plasma insulin by ELISA whereas the longer-term study analysed serum samples by RIA this difference in methodology is likely to have contributed, along with the different sampling methodologies, to the large differences observed in insulin concentrations between the acute and longer-term studies.
8.4 Overall conclusions

From the results of the systematic review, it can fairly confidently be stated that acute consumption of CC results in a temporary disruption to postprandial glucose metabolism. Furthermore, the results of the first study demonstrate that large doses of CC are not required and that this temporary disruption can be observed following a single serving of instant CC, such as is typically consumed in the UK. However, it is not known whether this effect is physiologically relevant as the magnitude of the effect is relatively small, with plasma glucose quickly returning to normal fasted levels in healthy individuals. There has been little research into the acute effects of CC in other groups, such as those with IFG and T2DM, where effects may be larger and clinically relevant. Furthermore, these studies have been carried out early in the morning after an overnight fast, when insulin sensitivity is high; these results cannot be extrapolated to later in the day when glucose tolerance declines. Outside of the laboratory setting, habitual coffee drinkers are likely to consume several cups of coffee throughout the day and will therefore have some caffeine in their system for most of the day. The effects of this habitual coffee consumption on the postprandial response to meals taken throughout the day is not known and should be investigated further.

There were no observed beneficial effects of increasing amounts of non-caffeine components on the postprandial response when consumed with 100 mg caffeine, suggesting that the amount of caffeine in a single serving of CC is sufficient to attenuate any beneficial effects of these other components. However, as discussed previously, 100 mg caffeine is towards the high end of the normal range, whereas the DC in this study contained relatively low amounts of the other components. Furthermore, it is probable that the study was underpowered to detect such a dose-response effect. Before drawing any conclusions from this data it would be advisable to repeat the study with a greater number of participants and a lower caffeine:CGA ratio.

In line with the systematic review, the second study found no effects of a single serving of DC, consumed in the morning, on the postprandial glucose and insulin response. This is in contrast to the literature observing a beneficial effect of CGAs when consumed in isolation, however it should be noted that the amount of CGAs consumed in these studies is much higher than that naturally found in a single serving of DC. Some interesting, albeit small, effects of DC consumed at lunchtime were observed, which may support the epidemiology reporting a reduction in T2DM risk associated with lunchtime coffee drinking. However, as this appears to be the first time this has
been investigated, and as the observed effects were small, further research is clearly required.

In the final study, there was little observed effect, beneficial or detrimental, of longer-term CC consumption in a group of coffee-naïve individuals, when the group was analysed as a whole. This is in contrast to Ohnaka et al. who observed some improvement in the postprandial glucose response following a similar intervention. However, their participants were older, with IFG; it is possible that the current study may have observed a similar effect in a less insulin-sensitive group. In the secondary analysis baseline differences were observed between participants with fast and slow caffeine-metaboliser phenotypes, with results suggesting that carriers of the slow allele may naturally have a less healthy overall metabolic profile. Furthermore, differences were observed between the two phenotypes in their response to the intervention, with both fast and slow phenotypes displaying some beneficial and some detrimental effects.

In conclusion, no strong evidence was found from either the acute studies or the longer-term study to support a preventative effect of coffee drinking on T2DM risk, as suggested by the epidemiology. There were however some interesting and conflicting effects in specific participant subgroups which warrant further investigation. It may be that coffee drinking is beneficial for some groups of people and not for others, however with little research to date in this area, it is clearly inadvisable to make any recommendations at this present time. It should also be remembered that the epidemiology only reports an association between coffee drinking and reduced incidence of T2DM. It cannot demonstrate a causal link. It is quite plausible that there is some other factor, unassociated with coffee drinking, and not corrected for within the statistics, that confers a reduced risk in this group.
8.5 Future work

Whilst producing some interesting results, these studies have also exposed further gaps in the literature which warrant study. Given sufficient time and funding the following additional investigations are suggested.

A repeat of Part B of the initial study, with the following modifications:

- Selection of instant DC based on its CGA content to ensure a higher total amount of CGA and/or addition of GCE to further enhance CGA content whilst maintaining a palatable drink.
- Addition of a reduced amount of caffeine, such as 40 mg, to further reduce the caffeine:CGA ratio and increase the possibility of observing a beneficial effect of the coffee, if one exists.
- Recruitment of a larger number of participants to increase the power to detect a dose-response effect.
- Repetition of study days to minimise intra-individual variation and increase the power of the study.
- Recruitment by genotype to establish whether fast and slow caffeine metaboliser phenotypes react differently to acute coffee consumption.

A crossover study investigating the acute effects of coffee consumption throughout the day in habitual coffee drinkers:

- One study day where the participants do not consume any coffee.
- A second study day where all meals and snacks are accompanied by a normal dose of instant CC.
- The study would be carried out under laboratory conditions, with timing and content of all meals carefully controlled.
- Postprandial glucose, insulin and lipid responses would be measured for all meals.
A repeat of the longer-term study with several modifications is advised:

- Recruitment by genotype and gender, with participants matched for bodyweight and body fat percentage to ensure an even balance between groups and reduce confounding.
- Recruitment of a larger number of participants to ensure subgroup analysis has sufficient power to detect any differences.
- Further analysis examining other SNPs known to be associated with caffeine metabolism as discussed in Chapter 7.
- Addition of two further study days to examine the acute effects of coffee in this group, both before and after the intervention.

A longer-term study investigating the effects of total coffee abstinence in habitual coffee drinkers may be of interest:

- Whilst it is not known how long it may take for any potential beneficial effects of regular coffee drinking to accumulate, it is also not known how long these potential benefits may take to disappear.
- Examining the effects of coffee abstinence may be a simpler way to investigate the longer-term effects of coffee drinking.
- It may also be easier to recruit coffee drinkers prepared to give up coffee for several weeks than it is to find non-coffee drinkers who are prepared to start drinking coffee regularly.

Finally, repetition of any/all of the above studies in people with IFG/T2DM would be of particular interest as little research has been carried out in these patient groups.


**Published work**

**Full Papers**


**Abstracts**

References


40. Kern PA, Ranganathan S, Li C, et al. (2001). Adipose tissue tumor necrosis factor and


Nutr. 81, 268S – 276S.


165. FSA. 2004. Survey of caffeine levels in hot beverages. Food Standards Agency;


Appendices

Appendix 1. Study consent form

Study name

- I the undersigned voluntarily agree to take part in the above named study.

- 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 expect to be able to comply with the requirements of the study as already outlined to me 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 £150. 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.

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 ........................................
Appendix 2. Medical and lifestyle questionnaire

Self-Certificate Medical Questionnaire

Name: ………………………………   Date of Birth: …………………………………..
Address: …………………………………………………………………………………………..
…………………………………………………………………………………………………..
Contact Telephone Number: ………………………………………………………………………

Please tick all/any of the following that apply:

☐ I have no prior/present history of Coronary Heart Disease, Angina or Stroke
☐ I have no prior/present history of Type 1 or Type 2 diabetes
☐ I have no prior/present history of anaemia
☐ I have no prior/current 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 endocrine disorders
☐ I have no prior/present history of, nor am I currently being treated for, clinical 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
☐ I am not currently taking or have taken any regular medication (including oral contraceptives) prescribed by my GP in the last 6 months (if you are please state what you are taking)
☐ I am not currently pregnant or breast-feeding

Have you been involved in a clinical trial in the last 3 months?   YES / NO
Are you currently on a weight-reducing diet or other dietary restriction?   YES / NO
If yes, please provide details.
Are you allergic or intolerant to any foods?   YES / NO
If yes, please state what foods you are allergic to.
Do you have any religious dietary requirements?  
YES / NO
If yes, please provide details.

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

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

<table>
<thead>
<tr>
<th>Alcohol</th>
<th>Measure</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ordinary strength lager (4%) e.g. Carling, Fosters</td>
<td>Pint</td>
<td>2.3</td>
</tr>
<tr>
<td>Strong lager (5.2%) e.g. Stella Artois, Kronenburg</td>
<td>Pint</td>
<td>3</td>
</tr>
<tr>
<td>Strong lager 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>
<td>3</td>
</tr>
<tr>
<td>Spirits</td>
<td>Std 25ml</td>
<td>1</td>
</tr>
<tr>
<td>Spirits</td>
<td>Lg. 35ml</td>
<td>1.4</td>
</tr>
<tr>
<td>Alcopop e.g. Smirnoff Ice, Bacardi Breezer, Reef</td>
<td>275ml</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Signed_________________________________________            Date___/___/___

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

-----------------------------------------------

Researchers use:

Fasted blood glucose:  ________________

Fasted haemoglobin:  ________________
Appendix 3. Regular caffeine intake questionnaire

Caffeine Questionnaire

Participant Number ________________ Date: ________________

Please answer the following questions as accurately as possible.

On average, how many of each of the following types of drink do you drink per week:

<table>
<thead>
<tr>
<th></th>
<th>Number per week</th>
<th>Size</th>
<th>With milk?</th>
<th>With sugar?</th>
<th>With sweetener?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Instant coffee (caffeinated)</td>
<td></td>
<td></td>
<td>YES/NO</td>
<td>YES/NO</td>
<td>YES/NO</td>
</tr>
<tr>
<td>Instant coffee (decaffeinated)</td>
<td></td>
<td></td>
<td>YES/NO</td>
<td>YES/NO</td>
<td>YES/NO</td>
</tr>
<tr>
<td>Filter coffee</td>
<td></td>
<td></td>
<td>YES/NO</td>
<td>YES/NO</td>
<td>YES/NO</td>
</tr>
<tr>
<td>Espresso-type coffee (includes espresso, cappuccino, latte etc)</td>
<td></td>
<td></td>
<td>YES/NO</td>
<td>YES/NO</td>
<td>YES/NO</td>
</tr>
<tr>
<td>Tea</td>
<td></td>
<td></td>
<td>YES/NO</td>
<td>YES/NO</td>
<td>YES/NO</td>
</tr>
<tr>
<td>Caffeinated cold drinks (eg cola, Red Bull)</td>
<td></td>
<td></td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>
### Appendix 4. Carbohydrate content of coffee used in 12 week intervention

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>(% w/w)</th>
<th>Amount per portion (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manitol (Free)</td>
<td>0.00</td>
<td>0</td>
</tr>
<tr>
<td>Fucose (Free)</td>
<td>0.00</td>
<td>0</td>
</tr>
<tr>
<td>Arabinose (Free)</td>
<td>0.98</td>
<td>20</td>
</tr>
<tr>
<td>Galactose (Free)</td>
<td>0.62</td>
<td>12</td>
</tr>
<tr>
<td>Glucose (Free)</td>
<td>0.29</td>
<td>6</td>
</tr>
<tr>
<td>Sucrose (Free)</td>
<td>0.00</td>
<td>0</td>
</tr>
<tr>
<td>Xylose (Free)</td>
<td>0.00</td>
<td>0</td>
</tr>
<tr>
<td>Mannose (Free)</td>
<td>1.93</td>
<td>39</td>
</tr>
<tr>
<td>Fructose (Free)</td>
<td>0.67</td>
<td>13</td>
</tr>
<tr>
<td>Manitol (Total)</td>
<td>0.00</td>
<td>0</td>
</tr>
<tr>
<td>Fucose (Total)</td>
<td>0.00</td>
<td>0</td>
</tr>
<tr>
<td>Arabinose (Total)</td>
<td>3.30</td>
<td>66</td>
</tr>
<tr>
<td>Galactose (Total)</td>
<td>16.20</td>
<td>324</td>
</tr>
<tr>
<td>Glucose (Total)</td>
<td>1.39</td>
<td>28</td>
</tr>
<tr>
<td>Sucrose (Total)</td>
<td>0.00</td>
<td>0</td>
</tr>
<tr>
<td>Xylose (Total)</td>
<td>0.09</td>
<td>2</td>
</tr>
<tr>
<td>Mannose (Total)</td>
<td>21.54</td>
<td>431</td>
</tr>
<tr>
<td>Fructose (Total)</td>
<td>0.08</td>
<td>2</td>
</tr>
</tbody>
</table>
Appendix 5. Product evaluation questionnaire

Study on the Effects of Different Coffees on Blood Glucose and Insulin

Participant No: ____________  Product: _______  Date: ____________

*Please circle the appropriate score*

1) On a scale of 1-5 how would you rate the overall palatability of the drink?

1  2  3  4  5

1 = unacceptable  5 = very acceptable

2) On a scale of 1-5 how would you rate the taste of the drink?

1  2  3  4  5

1 = unpleasant  5 = very pleasant

3) On a scale of 1-5 how would you rate the smell of the drink?

1  2  3  4  5

1 = unpleasant  5 = very pleasant

4) On a scale of 1-5 how would you rate the strength of the drink?

1  2  3  4  5

1 = weak  5 = very strong

*This study has received a favourable ethical opinion from the University of Surrey Ethics Committee.*
Appendix 6. Chlorogenic acid content of instant coffee (Kenco Decaff).

Data courtesy of Professor Alan Crozier and Dr Iziar Amaia Ludwig Sanz Orrio at the University of Glasgow.

<table>
<thead>
<tr>
<th>Rt</th>
<th>Compound</th>
<th>mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.64</td>
<td>CQA</td>
<td>0.29 ± 0.01</td>
</tr>
<tr>
<td>8.93</td>
<td>CQA</td>
<td>0.46 ± 0.01</td>
</tr>
<tr>
<td>9.55</td>
<td>3-CQA</td>
<td>5.87 ± 0.24</td>
</tr>
<tr>
<td>11.46</td>
<td>CQA</td>
<td>0.65 ± 0.04</td>
</tr>
<tr>
<td>12.00</td>
<td>CQA</td>
<td>1.25 ± 0.08</td>
</tr>
<tr>
<td>13.94</td>
<td>5-CQA</td>
<td>8.67 ± 0.28</td>
</tr>
<tr>
<td>14.70</td>
<td>4-CQA</td>
<td>6.28 ± 0.35</td>
</tr>
<tr>
<td>15.98</td>
<td>CQL1</td>
<td>0.36 ± 0.04</td>
</tr>
<tr>
<td>16.72</td>
<td>3-FQA</td>
<td>1.20 ± 0.05</td>
</tr>
<tr>
<td>20.79</td>
<td>CQL3</td>
<td>0.42 ± 0.03</td>
</tr>
<tr>
<td>22.26</td>
<td>CQL2</td>
<td>0.32 ± 0.03</td>
</tr>
<tr>
<td>22.99</td>
<td>CQL4</td>
<td>1.46 ± 0.09</td>
</tr>
<tr>
<td>24.34</td>
<td>CQL5</td>
<td>0.76 ± 0.03</td>
</tr>
<tr>
<td>25.59</td>
<td>5-FQA/4-FQA</td>
<td>3.00 ± 0.04</td>
</tr>
<tr>
<td>30.83</td>
<td>CQL6</td>
<td>0.06 ± 0.01</td>
</tr>
<tr>
<td>34.03</td>
<td>FQL1</td>
<td>0.08 ± 0.01</td>
</tr>
<tr>
<td>36.77</td>
<td>FQL2/FQL3</td>
<td>0.37 ± 0.04</td>
</tr>
<tr>
<td>38.08</td>
<td>FQL4</td>
<td>0.16 ± 0.03</td>
</tr>
<tr>
<td>44.20</td>
<td>3,4-diCQA</td>
<td>0.65 ± 0.05</td>
</tr>
<tr>
<td>44.85</td>
<td>3,5-diCQA</td>
<td>0.23 ± 0.02</td>
</tr>
<tr>
<td>50.51</td>
<td>4,5-diCQA</td>
<td>0.49 ± 0.02</td>
</tr>
<tr>
<td>65.00</td>
<td>diCQL1</td>
<td>0.02 ± 0.00</td>
</tr>
<tr>
<td>70.88</td>
<td>diCQL2</td>
<td>0.03 ± 0.00</td>
</tr>
<tr>
<td>71.93</td>
<td>p-Coumaroyl-tryptophan</td>
<td>0.08 ± 0.01</td>
</tr>
</tbody>
</table>

Total CQA: \[23.47 ± 0.97\]
Total FQA: \[4.21 ± 0.02\]
Total diCQA: \[1.38 ± 0.09\]

Total CQL: \[3.38 ± 0.04\]
Total FQL: \[0.70 ± 0.01\]
Total diCQL: \[0.05 ± 0.00\]

Data expressed as mean value ± standard deviation \((n=4)\) of 5-CQA equivalents. CQA: caffeoylquinic acids; CoQA: p-coumaroylquinic acids; FQA: feruloylquinic acids; diCQA: dicaffeoylquinic acids; CQL: caffeoylquinides/caffeoylquinic lactones; FQL: feruloylquinides/feruloylquinic lactones; diCQL: dicaffeoylquinides/dicaffeoylquinic lactones.