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**The effect of polyphenol-rich flaxseed muffins on glycaemic, insulinaemic and
24-hour glucose response in healthy adults**

By

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Declaration

I confirm that this is my own work and all materials that have been used in this work from other sources have been acknowledged.

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Abstract

Flaxseed may be considered a functional food as it contains chemical and bioactive compounds, such as antioxidants, polyphenols and dietary fibre. These compounds help to prevent diseases, such as cardiovascular disease (CVD) and Type 2 Diabetes (T2D), and they enhance metabolic health. It is important to investigate the use of flaxseed as a functional ingredient in bakery products to provide health benefits, particularly through its effect on blood glucose.

The main aim of this PhD was to evaluate the effect of flaxseed in different forms (whole and ground) incorporated into bakery products on the blood glucose profile of healthy individuals.

Ground flaxseed produced significantly higher levels of total polyphenols ($518.8 \pm 101.1 \mu\text{g GAE/g}$ compared to $260.1 \pm 25.7 \mu\text{g GAE/g}$ for the control muffins) and antioxidant capacity ($1693.9 \pm 78.6 \mu\text{mol/l}$ compared to $89.8 \pm 25.7 \mu\text{mol/l}$ for the control muffins). Both flaxseed forms reduced the rapidly digestible starch (RDS) in the muffins, which may lead to lower blood glucose levels.

Both flaxseed muffin types produced a significantly lower glycaemic response (GR) compared to standard glucose, but there were no significant differences between the flaxseed muffin types. Moreover, adding flaxseed lowered the Glycaemic Index (GI) value compared to the control muffins (64.3 ± 36.8 vs. 53.3 ± 13.2 and 42.2 ± 13 for the control, whole and ground flaxseed muffins respectively).

The results for the incremental area under the curve (iAUC) for glucose and insulin showed that both the whole and ground flaxseed muffins significantly lowered the GR

and insulinaemic response (IR) compared to the control muffins, at 60, 90 and 120 min ($p < 0.001$). There were no significant differences for the insulin iAUC 120 between the two flaxseed muffin types ($p > 0.05$).

The results of the blood glucose profile over 24 hours demonstrated that the flaxseed muffins were effective at lowering and maintaining blood glucose levels over 24 hours whether consumed once or three times a day. The results for the iAUC for the blood glucose were significantly lower for both flaxseed treatments during 24 hours ($p=0.014$) and during the night ($p=0.005$) compared to the control muffin.

Overall, the study found that flaxseed was effective amongst healthy individuals in relation to its effect on the glucose profile over 24 hours, gluco-regulation and insulin levels. These results add to the current body of evidence in the field of functional foods and support the use of such functional food ingredients in a healthy diet, specifically to improve the regulation of glucose in adults.

Table of Abbreviations

Abbreviation	Description
ALA	Alpha-Linolenic acid
AUC	Area under the curve
ANOVA	Analysis of variance
BMI	Body mass index
CGMS	Continuous Glucose Monitoring System
CHO	Carbohydrate
CM	Control muffins
CV	Coefficient of variation
CVD	Cardiovascular disease
DNS	Dinitrosalicylic acid
DPPH	2,2-Diphenyl-1-Picrylhydrazyl
DRVs	Dietary Reference Values
FAO	Food and Agricultural Organisation
FRAP	Ferric-ion Reduction Antioxidants Power
FCR	Folin-ciocalteu reagent
GAE	Gallic acid equivalents
GFM	Ground flaxseed muffins
GI	Glycaemic index
GL	Glycaemic load
GP	Gastric phase
GR	Glycaemic response
HDL	High-density lipoprotein
HbA1c	Glycated haemoglobin
iAUC	Incremental area under the curve
IR	Insulin response
ISO	International Organization for Standardization
LA	Linolenic acid
LDL	Low-density lipoprotein
PIS	Participant information sheet
RDS	Rapidly digestible starch
RS	Resistant starch
SEM	Standard error of mean
SD	Standard deviation
SDS	Slowly digestible starch
SDG	Secoisolariciresinol diglucoside
T2D	Type 2 diabetes
TG	Triglycerides
TPC	Total polyphenol content
USDA	U.S. Department of Agriculture
VAS	Visual analogue scale
WFM	Whole flaxseed muffins
WHO	World Health Organisation

Conversance and Publication

Almehmadi, A., Lightowler, H., Clegg, M. and Chohan, M. (2018) The effect of whole and ground flaxseed on glycaemic and insulinaemic response. *Proceedings of the Nutrition Society*, 77(OCE1).

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Chapter 1 : Literature Review

1.1 Introduction

Flaxseed (*Linum usitatissimum* L.) is one of the most ancient crops known to man. The Egyptians and Greeks used flaxseed from about 5000 BCE for medical purposes, especially for abdominal pains and as an energetic source of food, while flax fibre was also used as a material in clothing and paper. In the last two decades, flaxseed has become the focus of many studies of human consumption as a functional food because of its potential health benefits, especially in disease prevention (Oomah and Mazza, 1998; Singh *et al.*, 2011; Cardoso Carraro *et al.*, 2012).

Flaxseed was originally from west Asia and the Middle East. Regarding the production of flaxseed today, it is grown in more than 50 countries worldwide. Canada is the largest flaxseed producer, followed by China, the United States of America, India and Ethiopia. Canada produces almost 80 % of the global flaxseed crop (Rubilar *et al.*, 2010).

Flaxseed is a species of the Flax family, Linaceae, which has relatively short, fibrous, branched roots (90 - 120 cm). The flowers of this plant are hermaphrodite and hypogynous and are supported on long erect pedicels. They are made up of five blue petals, with five stamens, five sepals and five carpels separated by false septums, all of which make up the compound pistil; the capsule (fruit) may contain up to 10 seeds (Gill, 1987).

Flaxseed is described as a flat oval seed with a hard coat; the seed has a crisp and chewy texture with a nutty taste. Golden and brown flaxseed, two notable types of

flaxseed, are rich sources of fibre, lignans and omega-3 fatty acids (Freeman, 1995; Rubilar *et al.*, 2010).

1.2 Nutritional composition of Flaxseed

The analysis of the chemical composition of brown Canadian flaxseed averaged 20 % protein, 41 % fat, and 28 % dietary fibre based on Morris (2007) and Singh *et al.*, (2011). Comparing the nutritional composition of flaxseed to other oilseeds such as soya and sesame seed (Table 1.1), the dietary fibre is notably higher in flaxseed with 27.9 g /100 g compared to 6.1 g /100 g for soya and 7.9 g /100 g for sesame seed.

Table 1.1. Nutritional composition of flaxseed

Nutrient	Unit	Flaxseed*	Soya (boiled in unsalted water)	Sesame seed
Energy	kcal	492	141	598
Fat	g	34	7.3	58
Carbohydrate	g	34.3	5.1	0.9
Protein	g	19.5	14	18.2
Total dietary Fibre	g	27.9	6.1	7.9
Vitamin E	mg	0.3	1.13	2.53
Niacin	mg	1.4	2.7	10.4
Sodium	mg	34	1	20
Potassium	mg	681	510	570
Calcium	mg	199	83	670
Phosphorus	mg	498	250	720
Iron	mg	6.2	3	10.4
Magnesium	mg	362	63	370
Zinc	mg	0.6	0.9	5.3

Source: Food Standards Agency and Institute of Food Research (2002) * information is from US Department of Agriculture, Agricultural Research Service USDA(2003).

Regarding the nutritional composition for the two types of flaxseed (golden and brown), studies have shown that there was a variation between their nutritional composition. For example, Epaminondas *et al.* (2011) found a greater amount of soluble carbohydrates and a smaller amount of fibre in golden flaxseed compared to brown flaxseed, although proteins and lipids displayed no difference during their assessment of the chemical composition and antioxidant capacity of the seeds, which were rich in omega-3 and omega-6. Sargi *et al.* (2013), however, found there to be higher levels of omega-3 and omega-6 in golden flaxseed and higher antioxidant capacity in brown flaxseed.

The majority of studies in the literature have used ground flaxseed in the glycaemic control (Kapoor *et al.*, 2011) and assessing lipid profile (Parameshwari and Nazni (2012) as it is easier to digest through the human digestive system than whole flaxseed, which has soluble fibre, especially mucilage, found in the seed coat because their resilient seed coat resists the actions of digestive enzymes (Morris and Vaisey-Gener 2003). Whole flaxseed may not provide its full benefits as it is likely to pass through the digestive system, meaning ground flaxseed would produce greater health benefits (Morris, 2007). This has been highlighted by studies including that by Marambe *et al.* (2011) and Marambe, Shand and Wanasundara (2012) which compared the digestion of flaxseed protein in flaxseed meal (no mucilage) and whole flaxseed, and found the presence of mucilage reduced the digestibility of proteins *in vitro*, but there was no biological activity observed. Overall, the study could demonstrate conclusively that flaxseed biology is affected by flaxseed mucilage.

Processing of cereals can increase digestibility; studies have shown that effective methods are dehulling, germination and soaking (Oghbaei and Prakash, 2016). These

processes reduce the levels of tannins, polyphenols and phytic acid, which normally inhibit α -amylase activity and decreased starch digestibility. Large spaces can be created in the matrix due to tannin and phytic acid loss, meaning enzymes can act more readily, which in turn increases starch digestibility (Rehman and Shah, 2005).

Studies have shown that sieving or grinding can lead to a reduction in dietary fibre in flaxseed, but this processing can also increase digestibility and nutritional function, when compared to whole flaxseed (Morris, 2007). Flaxseed is crushed and ground to produce ground flaxseed, which can reduce the mucilage and crude fibre content and remove the hard seed coat (Dev and Quensel, 1988).

1.2.1 Macronutrients

The protein in flaxseed consists of 80 % globulins and 20 % glutelin (Goyal *et al.*, 2015). Flax amino acid pattern is quite similar to the protein of soybean (Oomah, 2001). However, it is not considered a complete protein reflected as a completed protein like soy because it does not contain enough of the essential amino acid, lysine (Oomah, 2001; Rabeetafika *et al.*, 2011). Amino acids in flaxseed contain comparatively more glutamic acid, arginine and aspartic acid and less cysteine and lysine (Ommah and Maza, 1993).

Flaxseed has been known for many years to have a high fat content. It is considered a rich source of polyunsaturated fatty acids, especially alpha linolenic acid (ALA), the essential omega-3 fatty acid and linoleic acid (LA), the essential omega-6 fatty acid, compounds which are essential in the diet as the body cannot synthesise these fatty acids itself (Morris, 2007).

The percentage of fats in flaxseed is around 30 %: 53 % ALA, 17 % LA, 19 % oleic acid, 3 % stearic acid and 5 % palmitic acid. Flaxseed can be an excellent source of omega-3 fatty acids, especially for people who have an allergy to fish (El-Beltagi, Salama and El-Hariri *et al.*, 2007).

Flaxseed oil is also low in saturated fatty acids (9 %), contains an average amount of monounsaturated fatty acid (18 %) and is high in polyunsaturated fatty acids (around 73 %). Flaxseed is considered to have an excellent ratio of omega-3: omega-6 fatty acids, which is around 3:1 (Goyal *et al.*, 2014; Strandvik, 2011). Although the high amount of ALA in the seed body can be extracted, this depends on the bioavailability, which is dependent on the form of the flaxseed. ALA in flaxseed oil has the highest bioavailability, followed by the milled flaxseed, while whole flaxseed has the lowest bioavailability (Austria *et al.*, 2008).

The main classification for the carbohydrates (CHO) divides them into two types according to their digestibility by the enzymes. The first class is those which are easily digested, and consists of simple sugars and starches; flaxseed contains very little of this type. The other class, which has resistance to the digestive enzymes, consists mainly of dietary fibre; most CHO in flaxseed is of the second type (Bhatty and Cherdkiagumchi, 1990; Vaisey-Genser and Morris, 1997).

Within the literature, there are discrepancies between the nutritional studies about the CHO content of flaxseed, and there are disagreements as to whether dietary consumption of this type of functional component can significantly contribute to total physiological nourishment (Dzuovor *et al.*, 2018). Generally, flax is considered to have quite low levels of sugars/starches or CHO (≈ 1 %); for flaxseed, however, the

carbohydrate content has been reported to be about 29 % (Bernacchia *et al.*, 2014; Kajla ,Sharma and Sood, 2014). These results could be part of the reason why flaxseed has not been not classified as an important source of CHO in the diet.

The discrepancy in the total carbohydrates may be due to different methods in the assessment of the total amount of carbohydrate. For example, in North America, conventionally total carbohydrate has been estimated by measuring fat, moisture, ash and protein and subtracting from the total weight of the dry food, the remainder being regarded as carbohydrate “by difference”. Assessment in Australasia and Europe is according to the sum of the individual carbohydrates (Mann and Truswell, 2012).

In food composition databases, evaluation of available CHO ‘by difference’ is a frequently used technique (Food Standards Agency, 2002). However, studies have indicated that the ‘difference’ technique is insufficient for consumers because the physiological impact of each carbohydrate fraction varies between the constituents (FAO/WHO, 1998; Greenfield and Southgate, 2003 ; Menezes *et al.*, 2009). Moreover, if carbohydrates are measured by this process, it may lead to an overestimated amount of available CHO, especially in food which is rich in resistant starch (RS) (Granfeldt *et al.*, 2006). In addition, experimental mistakes could cause inaccurate calculations. Such errors can involve any analytical techniques utilised to establish alcohol, ash, fat, fibre, protein and water. Furthermore, “by difference” does not permit a comprehensive characterisation of carbohydrates. However, this is essential for the comprehension of the association between health and carbohydrates as well as making claims (Mann and Truswell, 2012).

Therefore, it is necessary to measure carbohydrates directly, if accuracy is to be established. Colorimetric techniques may be utilised in order to assess carbohydrates (Dubois *et al.*, 1956). Two techniques are extensively applied to establish the content of carbohydrates in foods: phenol-sulphuric acid (PS) assay (Dubois *et al.*, 1956) and 3, 5-dinitrosalicylic acid assay (DNS) (Miller, 1959). It is possible to recognise all types of carbohydrates according to the colour, which develops as a result of the reaction of reagents and sugars. There is a connection between the intensity of the developed colour and the carbohydrates in the food samples. Colorimetric methods are regarded as being the most powerful system for quantifying and identifying carbohydrates.

Flaxseed, when compared to other whole grains, is considered a high source of dietary fibre. It contains both soluble and insoluble fibre with a ratio of soluble to insoluble varying between 20:80 and 40:60. Flaxseed has been shown to contain twice as much fibre as high fibre beans, with 14 g of whole flaxseed providing 20-25 % of the Dietary Reference Values (DRVs) for fibre (Mazza and Oomah, 1995; Morris, 2007; Qian, Cui and Goff, *et al.*, 2012). The soluble fibre in flaxseed is mucilage gum, while the insoluble fibre is cellulose, hemicellulose and lignin (Vaisey-Genser and Morris, 2003).

Mucilage gum is a soluble fibre that consists of polysaccharides that have the property of forming a gum solution when mixed with water or other fluids. It appears in the seed coat at 7-10 % of the seed's weight (Singh *et al.*, 2011). Within the flaxseed, the endosperm and the flaxseed coat form six layers; the outer layer of the hull contains the soluble gum flax mucilage, which is readily released when the hull is soaked in water (Daun *et al.*, 2003). The process of hydration makes the mucilage cells swell,

which allows their contents to be exuded onto the surface of the seeds. Previous studies into the effects of this were based on gum extracts from either flaxseed meal or whole seed (Qian, Cui and Goff, 2012). Studies into the properties of extracted mucilage have shown that there is variation in physicochemical properties, protein content and extraction yield, depending upon the variety of the raw material and the extraction process used. Flaxseed mucilage is composed of 4-20 % protein, 50-80 % carbohydrates, and also ash (Barbary *et al.*, 2009).

Lignin, which is generally found in woody plants, is among the strongest fibre types, and is another compound which is found in the cell wall of flaxseed (Morris, 2007). Lignin is composed of the phenyl groups form the polymers that compose the lignIn to form complex three-dimensional molecules of cross-linked phenol polymers, which are very important for the formation of cell walls, especially in bark and wood, as they are very rigid and do not rot easily (Lebo *et al.*, 2001). Within flaxseed, there are only small amounts of lignin in the flaxseed fibre, where it helps to strengthen the cross-linked microfibrils that form hemicellulose homopolymers (Scheller and Ulvskov, 2010).

Dietary fibre provides health benefits, especially in reducing heart disease, obesity and T2D (Anderson *et al.*, 2009). It also provides the feeling of fullness by bulking the gut, which has an effect on appetite, potentially aiding weight management as well playing a role in controlling the blood glucose level (Slavin and Green 2007; Russell *et al.*, 2016).

1.2.3 Micronutrients

Flaxseed is rich in minerals, especially calcium, magnesium and phosphorus; 30 grams of flaxseed can provide between 7-30 % of DRVs for these minerals (Goyal *et al.*, 2014). By measuring the concentrations of minerals in 5 g of milled flaxseed, the results showed the seeds contained 19 mg calcium, 34 mg magnesium and 66 mg of potassium (Daun *et al.*, 2003; Singh *et al.*, 2011).

There are many fat and water soluble vitamins in flaxseed such as thiamine, riboflavin, niacin and vitamin B-6 (USDA, 2017). There is an abundant presence of vitamin E in flaxseed mainly as gamma tocopherol (Bernacchia *et al.*, 2014). The presence of tocopherol in flaxseed can range from 8.5-39.5 mg in 100 g of whole flaxseed and 0.7-3.2 mg/tbsp in milled flax (Daun *et al.*, 2003).

1.2.3 Polyphenols

The name polyphenol is applied to a large and heterogeneous group of phenol ring containing phytochemicals (Hanhineva *et al.*, 2010). Studies have shown that there are several hundred different polyphenols in plant-based foods such as fruits (pears, grapes, cherries, apples, and different berries; these can contain 200 - 300 mg polyphenols per 100 g fresh weight), cereals, vegetables (particularly cabbages, broccoli and onions), legumes (such as soybean), chocolate, and plant-derived beverages (Scalber *et al.*, 2005; Pandey and Rizvi, 2009).

Polyphenols are present in flaxseeds in different forms such as phenolic acids, 1 %, flavonoids (0.35-0.70 mg in 1 g of seed) and lignans (1-26 mg in 1 g of seed). Studies have shown that the presence of lignans in flaxseed is 75-100 times greater than in

any other plant, making them a key source of plant lignans. The key lignan in flaxseed is secoisolariciresinol diglucoside (SDG) (Oomah, Kenaschuk and Mazza, 1995; Muir, 2006; Katare *et al.*, 2012).

1.2.3.1 Phenolic acids

Phenolic acids can be divided into two classes based on the acid they are derived from: hydroxycinnamic acid (from cinnamic acid) and hydroxybenzoic acid (from benzoic acid). Studies have shown that phenolic acids are an important component of flaxseed, as they can contain 3-5 g/kg of etherified phenolic acids and about 5 g/kg of esterified phenolic acids, which gives a total phenolic acids content of 8-10 g/kg (Oomah, Kenaschuk and Mazza, 1995). Within milled flaxseed flour, this converts to about 64-80 mg/tbsp of phenolic acids; a further study has shown that there are four key phenolic acids in this, being traces of 4-hydroxybenzoic acid, gallic acid (2.8 mg/g), chlorogenic acid (7.5 mg/g) and ferulic acid (10.9 mg/g), and of these trans-ferulic, trans-p-coumaric and p-hydroxybenzoic acids are ester bound (Oomah, Kenaschuk and Mazza *et al.*, 1995; Kasote 2013). Studies have also found caffeic acid and the related glucosides in flaxseed, with variations in the actual phenolic content depending on seasonal effects (Babrowski and Soulski, 1984; Oomah, Kenaschuk and Mazza, 1995).

1.2.3.2 Flavonoids

The total flavonoid content of flaxseed varies depending upon cultivar and growing conditions, and is usually in the range of 0.3-0.71 g per kg (Oomah, Mazza and Kenaschuk *et al.*, 1996). The flavonoids in flaxseed are found as their glucosides, including kaempferol 3, 7-O-diglucopyranoside, herbacetin 3, 7-O-dimethyl ether,

herbacetin 3, 8-O-diglucopyranoside, and herbacetin diglucoside (HDG) which is found ester linked via 3-hydroxy-3-methylglutaric acid (HMGA) in the lignan macromolecule (Qiu *et al.*, 1999; Struijus *et al.*, 2007).

1.2.3.3 Lignans

The richest source of plant lignans is flaxseed (Thompson *et al.*, 1991). Lignans are phytoestrogens; these are extensively present in fibre rich plants, cereals (wheat, barley, and oats), legumes (bean, lentil, soybean), vegetables (broccoli, garlic, asparagus, carrots), fruits, berries, tea and alcoholic beverages (Mazur *et al.*, 2000; Meagher and Beecher, 2000; Murphy and Hendrich, 2002; Hosseinian and Beta, 2009).

Secoisolariciresinol diglucoside (SDG) is the main lignan of flaxseed, which also contains lesser quantities of matairesinol, pinoresinol, lariciresinol and isolariciresinol (Meagher *et al.*, 1999; Sicilia *et al.*, 2003; Krajcova *et al.*, 2009) (Figure 1.1). The measurement of SDG generally ranges from 11.7-24.1 mg/g in defatted flaxseed flour and 6.1-13.3 mg/g in whole flaxseed flour (Johnsson *et al.*, 2000).

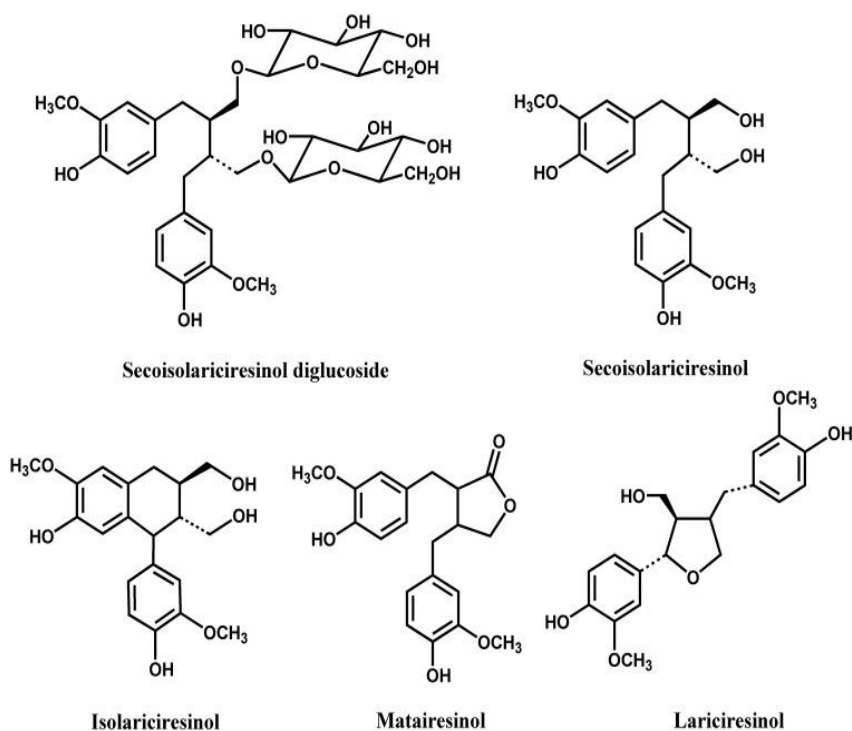


Figure 1.1. Chemical structures of the major lignans in flaxseed (Sicilia, *et al.*, 2003).

It has been shown that the flaxseed lignan SDG and the mammalian lignans enterodiol (ED) and enterolactone (EL) are effective as antioxidants against DNA damage and lipid peroxidation (Soni *et al.*, 2016). It is thought that the lignan antioxidant activity is related with the 3-methoxy4-hydroxyl substituents of SDG and secoisolariciresinol SECO. Indeed, SDG from flaxseed can delay the development of type 1 and type 2 diabetes (Touré and Xueming 2010). In addition, it has been argued that the hypoglycaemic effect of SDG in T2D occurs because of its antioxidant activity (Soni *et al.*, 2016).

1.3 Health benefits of flaxseed

Flaxseed is one of the oilseeds, which has been the focus of many studies from both nutritional and medical aspects, due to its chemical composition and bioactive

compounds, which have the potential to provide health benefits and prevent disease (Touré and Xueming 2010). It is considered to be one of the richest plant sources of lignan, which has been suggested to be able to lower the risk of CVD as well as inhibit the effect of developing T2D by reducing inflammatory response and insulin resistance (Mueller *et al.*, 2010).

1.3.1 Type 2 diabetes

The antioxidant effect of flaxseed is able to reduce plasma glucose and glycosylated haemoglobin (HbA_{1C}) concentrations (Cunnane *et al.*, 1993). Flaxseed may therefore have a role in reducing the risk factors of diabetes, obesity and dyslipidemia (Morisset *et al.*, 2009). Flaxseed contains both soluble and insoluble fibre, which plays a key role in reducing postprandial blood glucose levels (Qian, Cui and Goff, 2012). This is attained by reducing the release of sugar more slowly, which maintains blood glucose at the normal level for longer time periods (Thakur *et al.*, 2009).

Dietary fibres, lignan and omega-3 fatty acids contained in flaxseed have the ability to protect against diabetes by improving insulin sensitivity and glycaemic control (Nettleton and Katz, 2005; Karakas *et al.*, 2016). It has been demonstrated that flaxseed lignan SDG limits the expression of the phosphoenolpyruvate carboxykinase gene which is regarded as being the principal enzyme in glucose synthesis in the liver (Prasad, 2002). Fasting blood glucose was reduced by 19.7 % and HbA_{1C} by 15.6 mmol/mol by supplementing the diet of 29 people with T2D with 10 g of flaxseed powder for one month compared to the control group which who did not take flaxseed (Mani *et al.*, 2011). Although this may be attributed to the reduced content of high GI carbohydrates, the content of dietary fibre showed an increase as a result of the

flaxseed. Considerable research, utilising a fasting glucose tolerance method, has demonstrated a reduction in postprandial blood glucose levels of postmenopausal females consuming flaxseed (Cunnane *et al.*, 1993 and Cunnane *et al.*, 1995).

The effect of supplementation of flaxseed powder in menopausal females with diabetes was described by Kapoor *et al.* (2011). Ninety females were given 15 g and 20 g/day of flaxseed powder for two months, and as a result, postprandial blood glucose levels decreased by 7.9 and 19.1 % respectively; furthermore, the fasting blood glucose level showed a reduction in both flaxseed treatments by 6.4 % and 13 % respectively. Nazni, Amrithaveni and Poongodi (2006), who observed a decline in the blood glucose levels of 25 people with diabetes who had received supplemental 5 g of flaxseed powder in bread for 90 days, reported similar results. A study by Mohamed *et al.* (2012) confirmed the results reported by Nazni, Amrithaveni and Poongodi (2006). Mohamed *et al.* (2012) investigated the effect of flaxseed on blood glucose among participants with T2D and healthy participants (8 with T2D and 8 healthy) who consumed flaxseed bread supplemented with a 10 % defatted ground flaxseed replacement for the flour for eight weeks. The results for glucose indicated that adding flaxseed to the bread with 10 % replacement showed a significant reduction in glucose level in diabetic patients, whereas no significant change was detected in normal patients.

In diabetic patients, the decrease in the plasma glucose level was 14 % in comparison to baseline level. In diabetic patients, an appreciable reduction of 15 % in the postprandial glucose level was observed subsequent to supplementation of the dietary regimen with flaxseed bread in comparison with the starting baseline level. A study was conducted by Soltanian and Janghorbani (2018) on the impact of 10 g flaxseed in

biscuits among 53 T2D patients for a period of 12 weeks. The results for HbA1c and fasting blood glucose showed a reduction in the flaxseed group; however, the placebo group showed no changes. An insignificant reduction of approximately 0.8 % ($p=0.137$) was observed in the flaxseed group. From the baseline, there was a difference between average HbA1c and fasting blood glucose between the placebo and flaxseed groups (-26.7 vs. -1.9 mg/dl; $p = 0.011$ for FPG and -0.8 vs. 1.0 %; $p = 0.001$ for HbA1c).

Thakur *et al.* (2009) conducted a study on the impact of flaxseed on blood glucose among T2D participants by the addition of 5 % gum fibre to chapatti for a period of three months among 120 patients, which involved 60 each in the treatment and control groups. The outcome of their study conformed to the results shown above that flaxseed leads to an appreciable reduction of fasting blood glucose, the blood glucose falling decreasing from 154 to 136 mg/dl ($p=0.03$).

The aforementioned studies involved the application of flaxseed powder with in-bakery products or alone. However, another study on the impact of flaxseed lignan SDG has demonstrated that it is beneficial for T2D patients. In China, a randomised, double-blind crossover study was conducted among T2D patients in order to investigate the impact of a flaxseed-derived lignan supplement which comprised 360 mg SDG per day on indices of lipid profiles, insulin resistance and glycaemic control. The placebo and intervention time periods occupied 12 weeks, but were separated by an eight-week washout period, and 68 patients completed the trial. The lignan supplement led to an appreciable reduction in HbA1c concentrations in comparison with the placebo. However, no impact was observed upon fasting glucose and insulin

concentrations or the homeostasis paradigm evaluation of insulin resistance (HOMA-IR) (Pan *et al.*, 2007).

Contrastingly, a study was conducted by Dodin *et al.* (2008) among 179 non-diabetic menopausal women on the glycaemic response subsequent to the consumption of 40 g flaxseeds in various formats. These included 20 g added to bread and the remaining 20 g of ground flaxseed added to their daily diet, while the placebo group added wheat to the diet. The results showed that following the addition of flaxseed for a period of 12 months there was no appreciable difference concerning the glycaemic results, which disagreed with the results of the aforementioned studies. Furthermore, a study of 34 well-controlled diabetes patients conducted by Taylor *et al.* (2010) showed a similar outcome to the study undertaken by Dodin *et al.* (2008). In this study, for a period of 12 weeks each participant ate a variety of bakery products which contained one of the following: flaxseed oil, milled flaxseed or a control. In adults with well-managed T2D, no appreciable results were obtained concerning glycaemic control in either flaxseed treatment group, flaxseed oil or milled flaxseed. Lastly, a study was undertaken by Janidi *et al.* (2016) on the effect of the addition of 20 g or 40 g of flaxseed among 92 pre-diabetic patients for a period of 12 weeks. The results revealed there was no change in the glycaemic biomarker fasting blood glucose, IR as HOMA and beta- cell function, neither did the study obtained any notable results for either flaxseed treatment compared to the control group.

It is possible that the differences between these studies could be the result of assessments being conducted on different populations (type 2 diabetic patients vs. non-diabetic, male and female vs. female only). These studies used different forms of the flaxseed or its derivatives, and had different durations of the interventions and

sample sizes. This causes difficulty in reaching accurate conclusions on the effect on insulin sensitivity and glycaemic control of flaxseed and its derivatives based on these investigations (Mohammadi-Sartang *et al.*, 2018).

1.3.2 Cardiovascular diseases

It has been shown that the consumption of flaxseed, and more particularly ALA, may be advantageous to health. A study revealed that ingestion of 12 g of ALA as flaxseed oil capsules or flaxseed flour products in healthy women was effective in lowering blood lipids (Cunnane *et al.*, 1993). Furthermore, it was found that ground flaxseed containing a higher level of omega-3 fatty acids was effective in reducing cholesterol, triglyceride level and hypertension (Oomah and Mazza, 1998). This evidence implies that there may be a role for flaxseed in reducing cardiovascular risk factors (Parikh Netticadan and Pierce, 2018)

A meta-analysis conducted on 28 studies on the effect of flaxseed on lipid profile indicated that 20-50 g/d of flaxseed was significantly effective in reducing total cholesterol by between 0.1 and 0.2 mmol/L (3.87 to 7.73 mg/dL) and low-density lipoprotein (LDL)-cholesterol between 0.08 and 0.16 mmol/L (3.09 to 6.18 mg/Dl) among moderate hypercholesterolemic individuals (Pan *et al.*, 2009). Moreover, a study conducted by Jenkins *et al.* (1999) investigated the impact of flaxseed fibre on blood lipid profile among hyperlipidaemic patients. The participants were divided into two groups; one being a control group eating muffins containing fibre from wheat, the other being an experimental group whose consumed muffins containing 20 g flaxseed fibre for a period of three weeks. The results revealed that LDL-cholesterol levels were lower in the flaxseed group compared to the control group, while no change was

observed in high-density lipoprotein (HDL)-cholesterol levels. In another study, raw flaxseed was used at a dose of 50 g/day for a period of four weeks in which a reduction of 9 % in total cholesterol and 18 % in LDL was found in healthy participants (Cunnane *et al.*, 1995).

A study on the impact of 10 g flaxseed in cookies among 53 T2D patients for 12 weeks conducted by Soltanian and Janghorbani (2018) showed a reduction of cholesterol levels in a group who consumed flaxseed cookies ($p < 0.001$) for the course of the study; no reduction, however, was observed in the control group ($p=0.311$). Moreover, LDL reduced significantly for the flaxseed group ($p < 0.001$) compared to the control group ($p=0.482$); moreover in the flaxseed group there was an increase in HDL. These studies indicate that the different doses of flaxseed investigated have a measurable impact upon glucose and lipid levels.

Parameshwari and Nazni (2012) conducted a study on the effectiveness of flaxseed on lipid profile among hypercholesterolaemic patients; they were randomly allocated to a flaxseed group who received chapattis enhanced with 15 g of flaxseed or to a control group who received only their usual anti-hyperlipidaemic treatment for 4 weeks. The results are in agreement with the above studies that flaxseed improves the lipid profile by lowering total cholesterol, LDL and Triglycerides (TG) significantly ($p < 0.001$).

A study by Stuglin and Prasad (2005) disagreed with the above results because they found that flaxseed did not make any improvement in lipid profile; the study was among healthy participants who consumed flaxseed muffins with 32.7g of flaxseed for 4 weeks, the results finding that there was no significant changes in total cholesterol, LDL or TG. The different results of the effect of using flaxseed on the

lipids profile could be due to the variation of the flaxseed form used, participant characteristics (such as age, sex) and health status, that is whether they were healthy or had a metabolic disorder such as T2D or hyperlipidaemia (Pan *et al.*, 2009).

An interference with the bile acid metabolism is the most probable flaxseed action method in the reduction of lipid profiles. However, in this situation, intraluminal viscosity has the ability to obstruct micelle formation, and consequently reduce lipid uptake and inhibit re-uptake of bile acids. This would result in an increase of hepatic synthesis of bile acids, thereby redirecting cholesterol from lipoprotein synthesis within the liver, which would consequently reduce serum cholesterol (Theuwissen and Mensink, 2008; Kristensen *et al.*, 2012). This was attained by increasing the chime viscosity in the upper intestine which could subsequently lead to a reduction in the contact with digestive enzymes, thereby delaying absorption. Short-chain fatty acids are generated by the fibre fermentation within the intestine. This has proved to be effective in improving the peripheral insulin sensitivity (Robetson *et al.*, 2012).

1.3.3 Flaxseed and obesity

By reducing hunger and restricting the absorption of nutrients, dietary fibres obtained from flaxseed have a direct impact on health through the regulation of body weight (Kristensen *et al.*, 2012). To explore this issue, Mohammadi-Sartang *et al.* (2018) conducted a meta-analysis and systematic review of 45 randomised placebo-controlled trials that explored the impact flaxseed has on body weight and composition. The results indicated that using a whole flaxseed supplement for a period greater than 12 weeks in people whose Body Mass Index (BMI) was greater than 27 kg m² can lessen weight, BMI, and waist circumference. These findings align

with those of Soltanian and Janghroni (2018), who observed that providing 10 %g flaxseed to T2D patients for 12 weeks led to a significant reduction in body weight and BMI in the flaxseed group ($p < 0.001$), but not in the placebo group ($p = 0.947$ and $p = 0.930$). Specifically, there were differences in BMI and average weight loss between the two groups compared to baseline (-3.8 vs. 0.0 kg and -1.5 vs. -0.1 kg/m²; $p < 0.001$).

Conversely, research by Hutchins *et al.* (2013) on the effect of flaxseed on 25 obese participants yielded rather different findings. The participants in this study were organised into three groups: a group receiving a low dose of ground flaxseed (13 g), a group receiving a high dose of flaxseed (26 g for 12 weeks), and a control group receiving no flaxseed. The results showed there was no significant difference in either body weight or BMI compared to baseline for both treatment groups. These results can be explained by the fact that the flaxseed which was used in the study should be store at 0°C until use, If during the study the flaxseed was stored above 0°C for a long period of time, then this could have caused the active compounds to become unstable, which would alter the levels of biomarkers assessed in addition, this depends on the participants following these instructions, but as the study did not assess compliance of the participants it is not clear if they followed those instructions. Hutchins *et al.* (2013).

Ye *et al.* (2015) have postulated that body weight can be changed by consuming a diet high in fibre content and by stimulating the generation of satiety hormones. Soluble dietary fibre has been demonstrated to reduce body weight and thus obesity (Howarth, Saltzman and Roberts, 2001). Furthermore, soluble dietary fibre may prevent any future weight gain and stimulate further weight loss by delaying gastric

emptying as the ingestion of a substantial amount of water can cause people to feel full (Kristensen and Jensen, 2011). Thus, certain viscous fibres may lessen feelings of hunger (Wanders *et al.*, 2011; Kristensen *et al.*, 2013). The non-starch dietary fibres that comprise flaxseed mucilage are soluble hydrophilic substances with multiple branches, the viscosity of which can slow down gastric emptying and the absorption of nutrients from the small bowel (Kristensen *et al.*, 2013). This suggests that the consumption of flaxseed could be an effective dietary intervention to help prevent and reduce levels of obesity (Mohammadi-Sartang *et al.*, 2018).

1.4 Glycaemic index, glycaemic response and insulinaemic response

1.4.1 Glycaemic index

CHO can be divided into four groups according to the number of simple glucose units: they are classified as monosaccharides, disaccharides, oligosaccharides, and polysaccharides (Englyst and Englyst, 2005). This classification is not a reliable indicator of the effect of CHO on metabolic biomarkers; CHO can therefore also be classified according to their physiological effects and this can provide useful information about the health effects of having CHO in the diet (Jenkins *et al.*, 1981). For the above reasons the concept of the GI was introduced in 1981 by Jenkins *et al.* in order to provide a better understanding of the effects of CHO on the postprandial blood glucose level, rather than focusing on the chemical classification. The GI concept depends on ranking CHO in food according to the immediate effects on blood glucose levels. It can be determined from the iAUC of a test food with 50 g available CHO, and is recorded as a percentage of the glycaemic response for the same

participant to the 50 g standard food glucose or white bread (Wolever and Jenkins, 1986; Wolever *et al.*, 2003; Sun *et al.*, 2010).

1.4.2 Glycaemic response

GR refers to the post-prandial blood glucose response that occurs in situations where a food or meal that incorporates CHO is ingested. Available CHO is identified as the CHO in a food that is digested, absorbed and metabolised as CHO, and it is occasionally known as the net CHO or glycaemic CHO (expressed as the monosaccharide equivalent for optimal comparability between carbohydrates) (FAO, 2003).

1.4.3 Insulinaemic response

Insulin can be defined as a peptide hormone which is released by the pancreatic β cells. These cells sustain normal levels of blood glucose by controlling carbohydrate, protein and lipid metabolism, facilitating cellular glucose uptake as well as promoting cell growth and division by means of its mitogenic impacts (Wilcox, 2005).

There are three ways that insulin can help with the control of the postprandial glucose levels, primarily the insulin-sensitive cells of the peripheral tissues, such as the primarily skeletal muscle, receive a signal from the insulin, which increases their glucose uptake. The second method is the insulin causes the liver to promote glycogenesis, and finally there is simultaneous inhibition of the pancreatic cells by insulin which stops them from secreting glucagon, and this signals the liver to stop gluconeogenesis and glycogenolysis producing glucose. When these three steps are combined together they result in a reduction on blood glucose (Aronoff *et al.*, 2004).

It may be useful to evaluate insulin responses for the following two basic reasons: (1) to assist in the comprehension of the mechanism whereby glucose responses are changed; (2) to supply clinically helpful information for planning a diet (Wolever, 2006).

CHO intake affects insulin levels and there is a positive correlation between CHO intake and IR. IR is affected by many factors, including age, gender, BMI, gastric emptying and the releasing of gut hormones. IR also differs according to the types of participant groups, such as those with impaired glucose tolerance, T2D and overweight participants. (Saddler, 2011).

1.4.4 Factors that influence GI

The GI value for a food can be affected by many factors including the processing methods used, such as cooking; the different compounds in the food, such as protein, fat and dietary fibre; the type of CHO, and the proportion of amylose and amylopectin within the food (Philippou 2017) .

There have been several studies investigating the effect of fibre content on GI, but there is little agreement: Wolever (1990a) found GI value was associated negatively with insoluble fibre, Nuttall (1993) similarly found that when fibre was added to CHO, only fibres which were soluble had a lowering effect on the concentration of postprandial glucose. Several other studies found that there was no direct linkage between the GI of a food and the fibre content (Jenkins *et al.*, 1981), or no relationship between postprandial IR and fibre content of foods (Holt, Miller and Petocz 1997).

Studies have shown that the GI value was reduced after addition of fat or protein to foods containing CHO (Miller *et al.*, 2006). With the inclusion of protein in food, there is an increase in secretion of insulin and reduction in the postprandial glucose response (Gannon *et al.*, 1988; Holmstrup *et al.*, 2010). Moreover, foods with a high protein concentration are also known to increase the secretion of insulin without increasing glucose concentrations (Nuttall *et al.*, 1984). Adding fat to a food can also delay the gastric emptying, which reduces the CHO available for absorption and digestion in the small intestine, which consequently lowers the postprandial glucose response (Collier *et al.*, 1984; Welch *et al.*, 1987). This is further confirmed by other studies that have shown that addition of fat or protein (5-15 g) to CHO reduced the glycaemic response in normal contributors (Owen and Wolever, 2003).

Polysaccharides can be present in a branched form, described as amylopectin, or as a linear form, known as amylose (Lovegrove *et al.*, 2010). The ratio or proportion of amylose to amylopectin in the foods containing CHO can have an effect on the GI value. Amylose is digested slowly compared to amylopectin; consequently a greater ratio of amylose to amylopectin decreases the plasma glucose response of starchy foods. The GI value is increased when the ratio of amylopectin is higher because it is more easily hydroxylated than amylose, due to the differences in their molecular structures (Vanamelsvoort and Weststrate, 1992).

Foods containing CHO are affected by different processing methods including extrusion, flaking, rolling, grinding, peeling, crushing, pressing, canning, packing, storing and cooking, as these will affect the size of the starch grain particles as well as their integrity (Occana *et al.*, 1988). These processing methods make a portion of the CHO more accessible for digestion, as the large grains of starch in foods containing

CHO are broken down, meaning the amylopectin or amylose becomes accessible for hydrolysis, which can significantly alter the GI value of a food (Wolever, 1990a; Lunn and Buttriss, 2007).

The effect of cooking on starch gelatinisation, digestibility and resistant starch formation can be seen in the granules of starch which expand when heated or brought into contact with water due to the disturbance in the amylopectin and amylose arrangement through which water is enabled to diffuse into the granules (Alsaffar, 2011). When the granules are subjected to continuous heat, they will burst, thereby causing the release of the starch. This process, which leads to thickening of the solution, is referred to as starch gelatinisation. Furthermore, this procedure eases the access of amylolytic enzymes to their starch substrate. It is a procedure frequently used in cooking (Alsaffar, 2011).

The granular structure of starch is impacted by thermal processing, and the cooking process results in starch granule gelatinisation, together with a loss of crystalline structure, consequently leading to an increase in starch digestibility (Chung, Lim and Lim, 2006; Wang and Copeland, 2013). Therefore, from the perspective of processing, it is possible to modify the starch digestibility by regulating the thermal conditions of the moist heat which has been applied (Escarpa *et al.*, 1997; Giraldo Toro *et al.*, 2016; Miao *et al.*, 2010). Studies have shown a notable reduction in the SDS fraction and a small change in the RS fraction as well as a considerable increase in the RDS (Chung, Lim and Lim, 2006; Miao *et al.*, 2010). The insulin response, postprandial glucose and raw starch digestion are slower than those for gelatinised and partially gelatinised starches (Holm *et al.*, 1988; Thorne, Thompson and Jenkins, 1983).

Initially, the term RS was formulated by Englyst ,Wiggins and Cummings,(1982) in order to define a small fraction of starch which resisted hydrolysis through pullulanase and α -amylase treatment *in vitro*. RS is the starch which has not been hydrolysed after a period of 120 minutes of incubation (Englyst,Kingman and Cummings, 1992).

Englyst ,Wiggins and Cummings,(1982) suggested the principal categorisation according to the attributes of the starch and its environment in food. The categorisations are as follows:

- RS1 relates to physically inaccessible starches captured within the cellular matrix;
- RS2 indicates uncooked starch granules in foods, for example, banana or raw potato;
- RS3 is described as retrograded starch fractions which form following the cooking and storage of foods at low temperatures, or possibly even at room temperature;
- RS4 involves indigestible fractions within chemically modified starches.

The attributes of various types of resistant starch show the impact of processing and of gelatinisation on the starch structure as well as on starch granule properties. Digestibility is increased and starch gelatinisation occurs when starch is heated and a sufficient volume of water is present. When high-amylose starches are cooled, they retrograde and form crystalline areas which are inaccessible to enzymatic hydrolysis (Sharma, Yadav and Ritika, 2008).

Nevertheless, when high-amylose starches are compared with general starches, their gelatinisation and expansion decreases (Hallström *et al.*, 2011).

It is necessary to give separate consideration to the impact of processing on each type of RS. The reason for this is that the various types of resistant starch have different structures and compositions. In the case of RS1, digestive enzymes cannot hydrolyse the starch due to the fact that they cannot penetrate the materials of the cell wall (Murphy, Douglass and Birkett, 2008). Consequently, there is a necessity for the homogenisation or grinding of the grains to break down the cell structures and reducing the RS content (Murphy, Douglass and Birkett, 2008). RS2 exists in raw starchy foods and it is anticipated that there would be a reduction in resistant starch content in the course of the processing leading to starch gelatinisation (Sajilata, Singhal and Kulkarni, 2006).

From another perspective, when starches gelatinise and recrystallise, RS3 is formed; therefore, it is possible that resistant starch content could increase when those foods are subjected to more treatment (Murphy, Douglass and Birkett, 2008). Processing elements have an impact on resistant starch formation and content within processed food, except for the intrinsic properties of starch, which have an effect on the formation of RS. Examples of these properties are granular structure, the ratio of amylose to amylopectin, and crystallinity. It is known that retrogradation and starch gelatinisation are both affected by baking, extrusion cooking and other processes, and also that such processes have an impact on the resistant starch yield in completed food (Sajilata, Singhal and Kulkarni, 2006).

RS has an impact on the glycaemic index by affecting the rate of starch digestibility. Food that contains a higher RS is digested more slowly, leading to a slower glucose

release rate and consequently a lower GI in comparison with low RS foods (Englyst, Kingman and Cummings, 1992).

1.4.5 Classification of the GI of foods

GI is divided into three categories; high, medium and low. High GI foods are those foods which have a high rate of conversion of available CHO into glucose with subsequent absorption by the small intestine. Foods having a high GI have a value of greater than or equivalent to 70 if glucose is used as standard reference; (Brand-Miller *et al.*, 2009; Wolever *et al.*, 1991). Medium GI foods are those foods which have a value between 55 and 70 if glucose is used as standard reference (Brand-Miller *et al.*, 2009). Low GI foods are those foods which have a value below or equivalent to 55 if the standard reference is glucose; (Wolever *et al.*, 1991; Brand-Miller *et al.*, 2009). The CHO in low GI foods, such as beans, oats and lentils, is slowly converted to glucose and that results in a steady increase in the insulin and blood glucose responses (Granfeldt *et al.*, 2005; Kalergis *et al.*, 2005).

1.5 Flaxseed and glycaemic response

A meta-analysis and systematic assessment of 25 studies on the impact of flaxseed upon glycaemic control was undertaken by Mohammadi-Sartang *et al.* (2018). The blood glucose outcomes were shown within 30 comparisons derived from 24 studies, which included 1610 participants. It was demonstrated that the consumption of flaxseed led to a considerable reduction in the blood glucose level (Weighted Mean Difference, WMD 2.94 mg/dl; 95 % CI 5.31 to 0.56; $p = 0.02$).

Table 1.2 Summary of studies on the effect of flaxseed and glycaemic response

Study	Participants characteristics	Intervention	Results
Mani <i>et al.</i> (2011)	29 T2D patients	T2D patients randomly assigned to a flaxseed group involving daily supplementation with 10 g of flaxseed powder over a period of one month, and a control group.	Supplementation with flaxseed reduced fasting blood glucose by 19.7 % and HbA1c by 15.6 %.
Dah <i>et al.</i> (2005)	11 healthy subjects	15 g of flaxseed fibre was added to their bread as well as consumed control bread; the GR was determining for 2 hours.	The level of postprandial glucose was reduced by the addition of flaxseed to the bread.
Barre <i>et al.</i> (2008)	12 T2D patients	Using the double-blind system, the subjects were randomly assigned to groups consuming flaxseed oil capsules or safflower oil capsules for three months.	Insulin, HbA1c and fasting blood glucose did not change by the end of the trial.

Study	Participants characteristics	Intervention	Results
Hutchins <i>et al.</i> (2013)	25 obese, pre-diabetic patients	The participants were assigned into 3 groups in a randomised study. 1st group was the control. 2nd group had 13 g added flaxseed. 3rd group added 26 g flaxseed. The participants were asked to add flaxseed to their snack or meal. Flaxseed was consumed for 12 weeks,	Flaxseed decreased glucose and insulin and improved insulin sensitivity as part of a habitual diet in overweight or obese individuals with pre-diabetes.

1.6 Rationale and aims of study

This project is important because it investigated the use of flaxseed as a functional ingredient in bakery products to provide health benefits, particularly through its effect on blood glucose. There have only been a limited number of previous studies on the effect of flaxseed on blood glucose, especially in healthy participants. Most of the studies in the literature used flaxseed as a supplement to improve lipid profile, and most of the studies have been among diabetic, pre-diabetic and hyperlipidaemia patients rather than healthy subjects. As the main objective of the present study was to investigate if small changes in the daily diet could improve the GR, healthy participants of normal weight were selected to see if this treatment might potentially decrease the risk of developing chronic diseases. The ability to normalize nocturnal

glycaemia can help to reduce the concentration of fasting glucose, which could potentially reduce the risk of developing CVD in healthy people (Kristo *et al.*, 2013).

This study investigated the use of flaxseed in different forms (whole and ground) on GR/IR, whereas most of the previous flaxseed studies have only used ground or flaxseed flour. To the best of our knowledge, there are currently no studies that compare the effects of using whole and ground flaxseed in bakery products (for example in muffins) on GR and IR. It is important to compare these two forms of flaxseed and how they affect GR/IR, as they have slightly different chemical compositions, especially with regard to the amount of fibre, which may be hypothesised to have different effects on GR/IR.

This PhD also investigated the effect of flaxseed on the blood glucose profile using Continuous Glucose Monitoring System (CGMS) based on a previous study which had supported the role of flaxseed in lowering the glucose level. However, to the best of our knowledge no study has previously investigated the effect of flaxseed over the 24 h post consumption and investigated whether it is most effective when consumed once a day or more frequently, among healthy individuals.

The main aim of this PhD was to evaluate the effect of flaxseed in different forms incorporated into bakery products on the glucose profile of healthy individuals.

The predominant aims were achieved via the completion of the following studies:

- Study 1 - Determination of the Total Polyphenol Content (TPC), antioxidant activity and *in vitro* digestibility of the different types of muffins (whole flaxseed, ground flaxseed and control).

- Study 2 - Comparison of the Glycaemic Index (GI) of the different types of muffins (whole flaxseed, ground flaxseed and control).
- Study 3 - The effect of whole and ground flaxseed and control muffins on the glycaemic and insulinaemic response.
- Study 4 – The effect of a split portion of flaxseed on the 24-hour glucose response post consumption.

Chapter 2 : Determination of the total polyphenol content (TPC), total antioxidant capacity and *in vitro* digestibility of different types of muffins (controls, whole and ground flaxseed)

2.1 Justification and aims

The current increase in consumer demand for functional foods, especially those that contain more fibre, antioxidants and minerals, is due to increasing levels of awareness of the benefits of therapeutic nutrition (Goswami *et al.*, 2015). Flaxseed could provide additional health benefits if used as a functional food ingredient (Daun *et al.*, 2003). The specific components that can provide health benefits in flaxseed include lignans, ALA and dietary fibre (Hall, Tulbek and Xu, 2006).

Polyphenols are naturally occurring secondary plant metabolites that can be divided into four groups, the flavonoids, lignans, phenolic acids and stilbenes, and are mainly found in fruit, vegetables, cereals and beverages (Pandey and Rizvi, 2009). Flaxseed is the highest plant source of lignans and contains other important phenolic compounds, including flavonoids, tannins, phenylpropanoids and phenolic acids (Kasote, 2013). Different epidemiological studies have shown that consuming foods with a high polyphenol content daily can be beneficial and protect against diseases, including diabetes, cancer and CVD (Graf, Milbury and Blumberget *al.*, 2005). The majority of studies have used ground, defatted flaxseed to investigate antioxidant capacity and TPC; there have been no studies investigating whole, full fat flaxseed on antioxidant capacity and TPC, to the best of our knowledge (Anwar and Przybylski, 2012; Kaur and Kaur, 2018)

For nutritional purposes, starches can be grouped into three categories depending on the rate and extent of digestion: RDS, slowly digestible (SDS) and resistant starch (RS) (Englyst, Kingman and Cummings, 1992). RDS is starch that produces a rapid increase in the glucose level in blood after ingestion, SDS is starch that is digested slowly in the small intestine, and RS is not digested in the small intestine so it is classed as a type of dietary fibre, but it can be fermented in the large intestine (Chung, Shin and Lim, 2008). Studies have shown that sucrose and starch absorption, digestion and metabolism can be altered by the presence of polyphenols and their metabolites (Hanhineva *et al.*, 2010). For example, the sugar released from CHO rich foods has been shown to be reduced by polyphenols, which fits with studies that have shown uncooked grains such as wheat and rice are good sources of SDS, as they contain polyphenols which can inhibit digestive enzymes preventing starch breakdown (Zhang and Hamaker, 2009; Coe, Fraser and Ryan *et al.*, 2013). CHO digestibility is usually measured *in vivo* by monitoring blood glucose levels for 2-3 hours, after consumption of a test meal but there are suitable *in vitro* methods for measuring CHO digestibility at each phase of digestion (oral, gastric and intestinal) which can identify the proportion of SDS and RDS in a test food (Thondre *et al.*, 2010). These *in vitro* methods can help to explain the effects on GR seen *in vivo* (Englyst, *et al.*, 1999).

The aim of this study was to determine the TPC using Folin-Ciocalteu assay and antioxidant capacity by Ferric-ion Reduction Antioxidants Power (FRAP) assay of different muffin types: whole flaxseed, ground flaxseed and control muffins. The study also aimed to evaluate the effect of adding flaxseed to the muffins on *in vitro* starch digestion, and the sugars released by the different muffin types (control, ground and whole flaxseed). It was hypothesised that both flaxseed muffins are rich sources

of antioxidants and polyphenols as well as their fibre content in the extract and that may led to reduce the starch digestion from muffins. To the best of our knowledge, there are no data comparing TPC and total antioxidant capacity, *in vitro* starch digestion and sugars released in different forms of flaxseed (whole and ground) incorporated into bakery products such as muffins.

2.2 Methods

2.2.1 Chemicals

In this study, acetate buffer, sodium acetate trihydrate, glacial acetic acid TPTZ, HCl, FeCl₃ and FeSO₄ were used for the FRAP. Folin-Ciocalteu, sodium bicarbonate, gallic acid and acetone were also used in TPC Folin-Ciocalteu assays. Pepsin from porcine gastric mucosa (P7000- ≥ 250 units/mg),pancreatin from porcine pancreas powder (P7545 >100 U/mg),amyloglucosidase(A7095- ≥ 260 U/ml), α -amylase (86250 ~ 1.5 U/mg), bile extract porcine and dinitrosalicylic acid (DNS) for *in vitro* digestion and estimation of sugar release. All of these chemicals were purchased from Sigma–Aldrich (Poole, UK).

2.2.2 Muffin preparation

Muffins ingredients (melted unsalted butter, self-rising flour, bicarbonate of soda, baking powder, ground cinnamon, nutmeg, caster sugar, bananas, eggs and semi skimmed milk) were purchased from a local supermarket Tesco, Oxford, UK). Whole and ground flaxseed was bought from a health food shop (Holland and Barrett, Oxford, UK).

The muffins were prepared following a recipe from the BBC Food (2018) and the flaxseed muffins were prepared by replacing 48 % of the flour in the control muffins with whole or ground flaxseed each flaxseed muffins contain 15 g whole or ground flaxseed and 16.25 g flour. All the ingredients which were required for each muffin can be seen in (Table 2.1). To prepare the muffins, first the dry ingredients (flour, sugar, baking soda, baking powder, nutmeg and cinnamon) were sifted and thoroughly mixed together with whole or ground flaxseed if required. The banana was mashed and the liquid ingredients (semi-skimmed milk, butter, vanilla and eggs) were mixed in before adding to the dry ingredients. All muffin mixtures were placed into non-stick petite muffin cups tray and baked at 190°C (375°F) in a pre-heated oven for 20 min. After baking, the muffins were allowed to cool for 10 minutes before they were then removed from the tray. Then they removed from the tray for complete cooling on a wire rack for 30 minutes. The muffins were stored in an airtight container overnight in order to analysis them next morning.

Table 2.1. Muffin formulation (g) for eight muffins (control, whole flaxseed and ground flaxseed)

Ingredients	Unit	Muffin formulation		
		Control	Whole flaxseed	Ground flaxseed
Melted butter	g	75	75	75
Self-rising flour	g	250	130	130
Flaxseed	g	-	120	120
Baking powder	g	5	5	5
Bicarbonate of soda	g	2.5	2.5	2.5
Ground cinnamon	g	2.5	2.5	2.5
Nutmeg	g	2.5	2.5	2.5
Caster sugar	g	115	115	115
Vanilla extract	ml	5	5	5
Large ripe bananas	g	240	240	240
Medium eggs	g	106	106	106
Semi skimmed milk	ml	125	125	125

A 150 g sample of each muffin type was analysed for nutritional content (Table 2.2) by Eurofins Scientific Food Testing Ltd. (Wolverhampton, UK). The methods of the analyse the composition of muffins can be found on Appendix 1.a.

Table 2.2. Nutrient profile for each muffin type.

	Control		Whole flaxseed		Ground flaxseed	
	/100g	Per muffin (116 g)	/100g	Per muffin (116 g)	/100g	Per muffin (116 g)
Crude protein (g)*	5.5	6.38	6.4	7.4	7.2	8.4
Carbohydrate (avail) (g)*	40.9	47.4	30.4	35.3	30.8	35.7
Total sugars (g)*	18.7	21.6	18.5	21.46	18.2	21.1
Starch(g)**	20.4	23.7	11.1	12.9	13.3	15.4
Total fat (g)*	8.5	9.7	15	17.4	14.9	17.3
Total fibre (AOAC) (g)*	1.6	1.9	5.6	6.5	4.9	5.7
Energy (kcal)*	265	307.4	293	339.9	296	343.4
Energy (kJ)*	1120	1299.2	1230	1426.8	1240	1438.4
Saturated fatty acids SFA (g)*	4.8	5.6	7.1	8.236	6.9	8.00

*Nutritional analysis was carried out by Eurofins Scientific Food Testing Ltd. UK.

** Nutrition information was calculated based on the recipe using Nutritics software.

2.2.3 Sample Preparation and Extraction

The different types of muffins (control, whole and ground flaxseed muffins) were crushed and approximately 1g was weighed into amber flasks to prepare the samples for extraction and analysis. Four ml of the extraction solvent (70 % acetone and 30 % water) was added to the samples, which were shaken for 2 hours at 200 room temperature at 25°C using Shaking Incubators (The Mickle Laboratory Engineering Co., Gomshall Surrey, England).

The samples were then centrifuged at $3000 \times g$ for 10 minutes (Heraeus Instruments, Kendro Laboratory Products, D-37520 Osterode, Germany) and the supernatant was

pipetted out and stored at -20°C until needed in the assays (Thondre, Ryan and Henry, 2011).

2.2.4 Ferric-ion Reducing Antioxidant Power FRAP Assay

After extraction, the FRAP assay was used to measure the antioxidant capacity in the sample using reduction of ferric tripyridul triazine (Fe^{3+} TPTZ) to ferrous form (Fe^{2+} TPTZ) the assay was adapted from the method by (Benzie and Strain 1996). The FRAP reagent was made by mixing reagent A:300 mM acetate buffer pH 3.6 (3.1g sodium acetate trihydrate and 16 mL glacial acetic acid made up to 1L dH₂O), reagent B:10 mM TPTZ in 40 mM HCl(3.4 mL HCl made to final volume 1 L dH₂O) and reagent C:20 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (5.406 g iron chloride made to final volume 1L dH₂O in a 10:1:1 ratio. The standard curve was produced by diluting a solution of 1000 μM $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$. One ml dH₂O was placed into test tubes and incubated in a water bath at 37°C for 5 minutes. Twenty-five μl of the sample or standard was then added and mixed vortex using IKA vortex (IKA Vortex 3, IKA Laboratory Equipment ,Germany) followed by addition of 1 ml of fresh FRAP reagent, mixed and incubated in the water bath at 37°C for exactly 4 minutes. The absorbance of the sample or standard was read at 593 nm using a spectrophotometer (Shimadzu UV-1800, Shimadzu UK Limited, Milton Keynes Buckinghamshire, UK).

The FRAP value was calculated using the following equation:

$$\text{FRAP for the sample} = \frac{\text{Observation of the sample}}{\text{Observation of the standard}} \times \text{FRAP for the standard}$$

FRAP value of 1000 μM $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 1000 μM .

2.2.5 Total Polyphenol Content, Folin-Ciocalteu FCR Assay

TPC assay uses the change in colour of Folin-Ciocalteu reagents as an indicator of polyphenol content (Prior, Wu and Schaich, 2005). Immediately before the assay, 1.5 ml of Folin-Ciocalteu reagent (diluted with water dH₂O in a ratio of 1:10) was added to 200 µl of the sample. After 5 minutes incubation, 1.5 ml of the second reagent (sodium bicarbonate solution 60 g/l) was added and reaction was incubated in the dark for 90 minutes. The absorbance was then measured at 725 nm using a UV-Vis Spectrophotometer (Shimadzu UV-1800, Shimadzu UK Limited, Milton Keynes Buckinghamshire, UK)

The standard used for this assay was gallic acid, prepared by dissolving 10 mg of gallic acid in 10 ml of 70 % acetone to 30 % water. The results of the total polyphenol content were presented as gallic acid equivalents (GAE) per gram of samples (Thondre, Ryan and Henry, 2011).

2.2.6 *In Vitro* Digestion

To stimulate human digestion, an *in vitro* digestion model was used, which contained two phases of digestion (gastric and ileal) with sampling prior to and post-gastric phase, and during the ileal phase at 20, 60 and 120 min (Mishra, Monro and Hedderley, 2008).

The *in vitro* digestion was carried out based on the method of Coe, Fraser and Ryan (2013); 2.5 g of each muffin type (control, whole and ground flaxseed) was placed in plastic pots, 30 ml of water was added. The 0 min sample was taken by adding 250 µl from each muffin type to a test tube containing ethanol (1:4 ratio). Next, 0.1 ml 10 %

α -amylase, 0.8 ml 1 M HCl, and 1 ml 10 % pepsin in 0.05 M HCl were added to each muffin sample. The muffin samples were then incubated at 37°C and stirred slowly (130 rpm every 15 s) for 30 min to finish the gastric digestion stage. A sample was taken at the end of the Gastric Phase (GP). To start the ileal stage, 2 ml 1 M NaHCO₃ and 5 ml 0.2 M sodium maleate buffer (PH 6) were added to each muffin sample with 55 ml dH₂O. In quick succession, 0.1 ml of amyloglucosidase and 1 ml of 2 % pancreatin solution (in maleate buffer, PH 6) were added to each muffin sample and they were incubated for 120 minutes with slow mixing. Samples of 0.25 ml were taken during the ileal stage at 20, 60, and 120 minutes. These samples were then centrifuged (1000 $\times$ g, 2 min) in a Biofuge Primo Centrifuge (Heraeus Instruments, Kendro Laboratory Products, Germany), and an aliquot of the supernatant was removed for reducing sugars analysis.

2.2.7 Analysis of Reducing Sugars Released during Digestion

To detect released sugars after *in vitro* digestion of the muffins a colorimetric method adapted from Englyst and Hudson (1987) was used. This analysed monosaccharides after treatment with amyloglucosidase as secondary digestion compound, as this produces depolymerisation of starch fragments. An amount of 0.05 ml of 10 mg/ml of standard glucose or 0.05 ml of each muffin sample was added to 0.25 ml of enzyme solution A (1 % amyloglucosidase in acetate buffer, PH 5.2) and incubated at 25 °C for 10 minutes. 0.75 ml of DNS mixture (0.5 mg/ml glucose: 4 M NaOH: DNS reagent mixed in a 1:1:5 ratio) was then added to all samples, which were incubated in a water bath at 95°C for 15 minutes. Three ml of dH₂O was then added and the samples were cooled in a cold-water bath for 20 minutes. The absorbance was then read at 530 nm on a spectrophotometer (Shimadzu UV-1800, Shimadzu UK Limited ,

Milton Keynes Buckinghamshire, UK) and sugar release was measured as mg per g of the muffin sample (Mishra and Monro, 2009; Coe, Fraser and Ryan, 2013). SDS was calculated as the difference between the sugars released at 120 and 20 minutes of digestion.

2.2.8 Statistical Analysis

Data were analysed using SPSS (Statistical Package for the Social Sciences) v25.0 (Chicago, IL, USA). The results were in triplicate and are expressed in terms of mean and standard deviation and standard of error. The Shapiro-Wilk test was performed to investigate the normality of the data; where data were not normally distributed, non-parametric tests were used. Based on the normality tests One-way between group ANOVA followed by Tukey for pairwise comparisons and Kruskal Wallis test were used to compare TPC and antioxidants between the three types of muffin. The results for the control, whole and ground flaxseed muffins at 0, GP and 20 min stages of the *in vitro* digestion were not normally distributed. Therefore, a Kruskal Wallis test was performed to compare all three muffins. One-way between groups ANOVA was used to compare the different muffins results for 60 and 120 min. The correlation between FRAP and TPC was assessed using Pearson's correlation. Statistical significance was set at ($p < 0.05$).

2.3 Results:

2.3.1 Total polyphenols Content TPC

The results for the TPC ($\mu\text{g GAE/g}$) for the different types of muffins (control, whole and ground flaxseed) (Figure 2.1) indicated that both forms of flaxseed muffins had a

significantly higher ($p < 0.05$) polyphenol content compared to the control muffins. The difference was also significant ($p < 0.05$) between the flaxseed muffin types, with ground flaxseed containing a higher concentration of polyphenols.

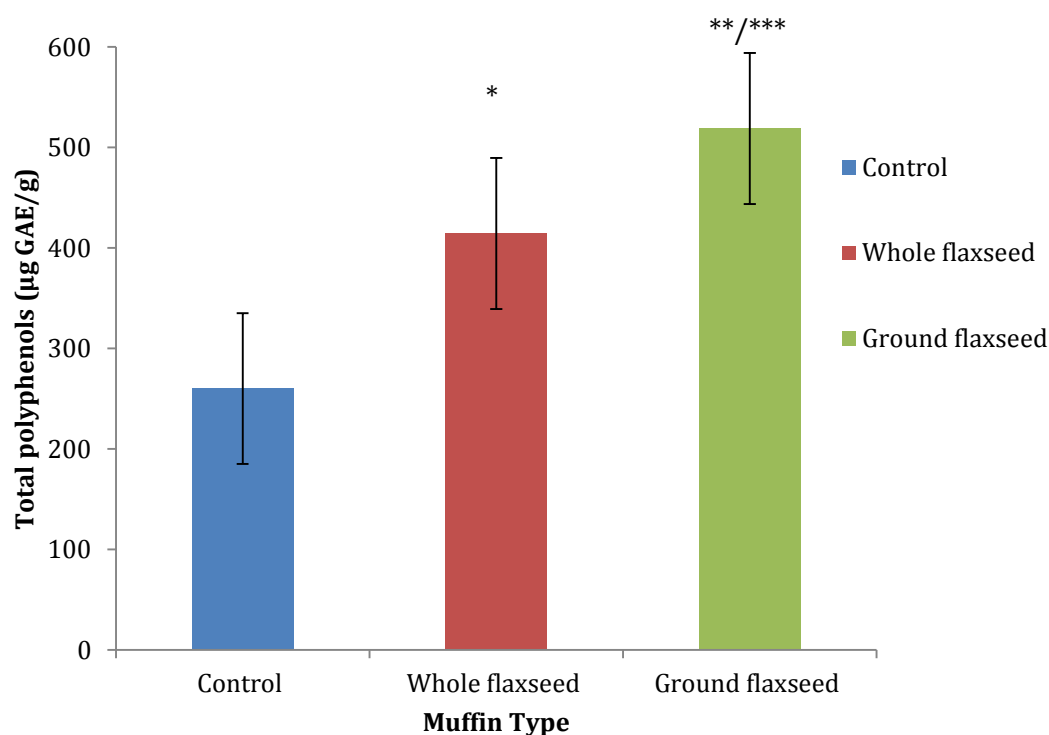


Figure 2.1. Total polyphenol content (µg GAE/g) for each muffin type

Values represent mean $\pm$ SEM of three independent experiments

*Significant difference ($p < 0.05$) between whole flaxseed muffin and control muffin

**Significant difference ($p < 0.05$) between ground flaxseed muffin and control muffin.

*** Significant difference ($p < 0.05$) between ground flaxseed muffin and whole flaxseed muffin .

2.3.2 Antioxidants capacity

Total antioxidant capacity was measured using FRAP (µmol /l) (Figure 2.2) and showed that ground flaxseed muffins had the highest capacity, followed by whole flaxseed and control muffins. These results were significantly different ($p < 0.05$) between the control muffins and both flaxseed muffins and between both flaxseed

muffins (whole and ground). These results are consistent with the results of TPC and both the results for polyphenols and antioxidants showed that flaxseeds in bakery products (muffins) had high polyphenol and antioxidant capacity.

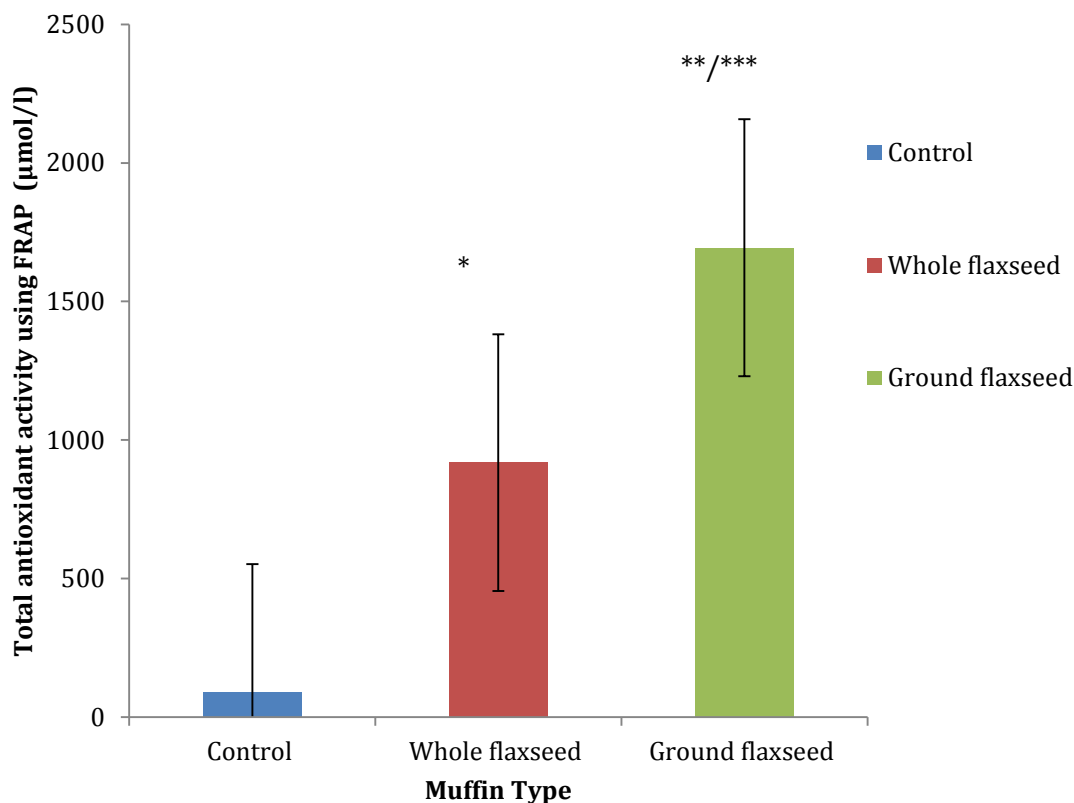


Figure 2.2. Antioxidant capacity using FRAP (μmol/l) for each muffin type.

Values represent mean ± SEM of three independent experiments.

FRAP= Ferric-ion reducing antioxidant power.

*Significant difference($p < 0.05$) between whole flaxseed muffin and control muffin.

**Significant difference($p < 0.05$) between ground flaxseed muffin and control muffin.

*** Significant difference($p < 0.05$) between ground flaxseed muffin and whole flaxseed muffin.

2.3.3 Sugar release

The results for sugar released during *in vitro* ileal digestion of the control and of two flaxseed muffins (Figure 2.3), as percentage of sugar realised per starch for each muffin type, showed the sugar release in both flaxseed muffins was significantly lower ($p < 0.05$) than in the control muffin. RDS during the first 20 minutes was

highest in the control muffins, and then in the whole flaxseed muffins and then the ground flaxseed muffins, which had the lowest. The difference between the control and the ground flaxseed muffins was significant ($p < 0.05$), but there was no significant difference between the flaxseed muffin types at this time point, while the control muffins released higher concentrations of sugar compared to both flaxseed muffin types. At 60 minutes this difference was significant ($p < 0.05$) between the ground and the whole flaxseed and the control muffins, while at 120 minutes the difference was not significant between all muffin types.

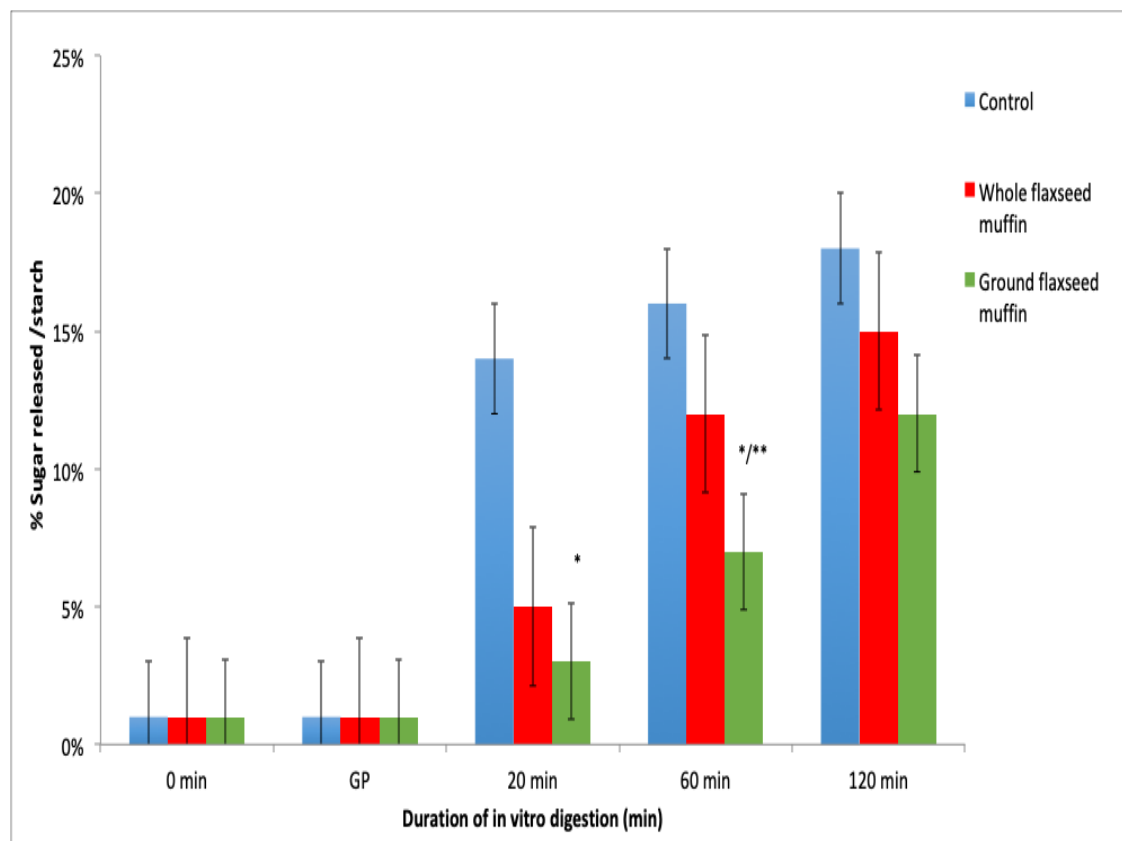


Figure 2.3. Percentage of sugar realised per starch for each muffin type.

Values represent mean $\pm$ SEM of three independent experiments.

20, 60 and 120 min = stages of intestinal digestion, GP = gastric phase.

*Significant reduction in sugar release ($p < 0.05$) between whole flaxseed muffin and control muffin.

**Significant reduction in sugar release ($p < 0.05$) between ground flaxseed muffin and whole muffin.

The results of SDS (Table 2.3) showed there were no significant differences, but the results were lowest for ground flaxseed muffins, while the control muffin was the highest which may be due to the higher amounts of sugar released at 120 minutes, as SDS is the sugar released between 20 and 120 minutes of digestion (Figure 2.3).

Table 2.3. Rapidly digestible starch (RDS) and slowly digestible starch (SDS) in control muffins and different types of flaxseed muffins

Muffin type	RDS (mg/g sample)	SDS (mg/g sample)
	Mean	Mean
Control	291.5	76
Whole flaxseed	60.6*	107.7
Ground flaxseed	42.3 *	112

Values represent mean of three independent experiments.

*Significance level $p < 0.05$ compared to control muffin.

RDS = sugar released after 20 min of digestion

SDS = sugar released between 20 and 120 min of digestion

2.3.4 Polyphenols and antioxidants correlation

The results (Figure 2.4) showed there was a strong positive correlation between the antioxidant capacity measured using FRAP ($\mu\text{mol/l}$) and TPC ($\mu\text{g GAE/g}$) for the 27-paried readings for all muffin types (control, whole and ground flaxseed muffins) ($r^2 = 0.63$, $p < 0.001$).

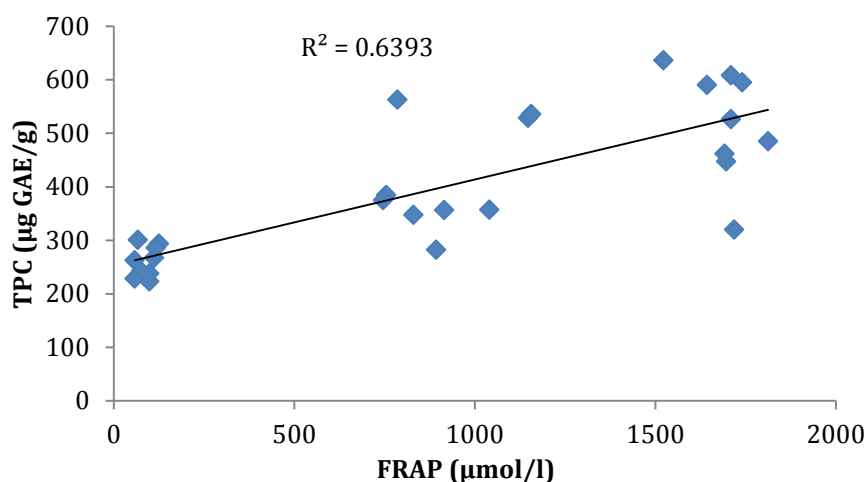


Figure 2.4. Pearson regression of 27 paired reading between the antioxidants capacity using FRAP ($\mu\text{mol/l}$) and TPC ($\mu\text{g GAE/g}$).

2.4 Discussion

Adding flaxseed enhanced the TPC and antioxidant capacity of the muffins, as well as prolonging the time for sugar to be released, for both flaxseed forms. However, ground flaxseed had significantly higher levels of TPC and antioxidant capacity compared to the whole flaxseed and control muffins. The results for sugar release indicated that both flaxseed forms were significantly different to the control, with a later sugar release; however, there was not any significant difference between the forms of flaxseed.

The results for TPC in this study fits well with the results of Kaur, Singhand Kaur *et al.* (2017), who found that replacing wheat flour with different percentages of flaxseed flour (5-10 %) enhanced the TPC and antioxidant capacity in cookies compared to control cookies without added flaxseed. The results on cookies showed a TPC of 19.32 mg GAE/100 g for the cookies with 30 % of flaxseed flour, compared to 8.23 mg GAE/100 g for the control cookies, which is similar to the trend seen in

our study: flaxseed muffins with 48 % of whole and ground flaxseed were higher in polyphenols with 414.4 and 518.8 $\mu\text{g GAE/g}$ respectively compared to the control muffins. The different results could be explained by the different flaxseed forms (whole and ground) in our study compared to the use of flaxseed flour in Kaur Singh, and Kaur *et al.* (2017), as well as by the percentage of flaxseed added to the product: the highest percentage of flaxseed flour in Kaur, Singh and Kaur *et al.* (2017) was 30 % whereas in the current study flaxseed was added to the muffins up to 48 %. Another reason for the observed differences could be the different products used (muffins vs. cookies).

The study by Kaur and Kaur (2018) agrees with the current study by adding flaxseed increased the amount of polyphenols and antioxidants in muffins using three different cultivars of flaxseed from India: Jawahar-27 from Madhya Pradesh, LC-2023 from PAU, Ludhiana; and Jeevan from CSKV, Palampur, Himachal Pradesh. The muffins were prepared by replacing wheat flour with 10-40 % of flaxseed powder from the three different cultivars. The results for Jawahar-27 with 40 % replacement which was the highest among flaxseed cultivars at 67.91 $\mu\text{g GAE/g}$ compared to 414.4, 518.8 $\mu\text{g GAE/g}$ for whole and ground flaxseed muffins (respectively) seen in our study. The different results between these studies could be due to the preparation of the muffins, since in Kaur and Kaur (2018) the flaxseed was ground and defatted using hexane, dried at 40°C for 24 h then extracted with methanol at room temperature for 60 min while in the current study the extraction was carried out by using 70 % acetone and none of the muffin samples was defatted. The current study also used whole or ground flaxseed rather than using flaxseed powder, which will have had an effect on the polyphenol and antioxidant content.

Brodowska *et al.* (2014) compared the antioxidants in defatted and non-defatted flaxseed. The results showed that non-defatted flaxseed extracts were higher in antioxidants compared to the de-fatted extracts, which suggests that the process of defatting flaxseeds using hexane as the solvent following the Soxhlet method can lower the natural antioxidant capacity such as vitamins A, E and C. Increasing the extraction temperature in the defatting process seemed to cause reduction and breakdown in the antioxidant content (Hossain *et al.*, 2013). Generally, studies have used de-fatted flaxseeds for their investigations, but as degreasing can affect antioxidant capacity and other studies have shown non-defatted flaxseeds contain higher levels of antioxidants, this would suggest that it would be beneficial to study flaxseeds as full fat whole seeds. The use of full fat flaxseeds in foods needs further investigation as their inclusion in the diet as novel foods could produce benefits to human health (Brodowska *et al.*, 2014 ; Nandi and Ghosh, 2015).

Most of the studies in the literature were carried out using defatted flaxseed, not many studies used non-defatted flaxseed. Full-fat flaxseed has been shown to be higher in polyphenols compared to defatted flaxseed; for example, a study carried out Alu'datt *et al.* (2013) explored the phenolic compound content in these flaxseed types and found 4.69 mg/g for full-fat and 3.60 mg/g for defatted flaxseed.

In this study flaxseed, which was non-defatted and which was used in different forms (whole and ground), displayed special properties regarding polyphenol type as the non-defatted flaxseed contained more soluble free phenolic acids, which make up more than 50 % of the total phenolic compounds in it. The soluble free phenolic acids have the ability to produce higher antioxidant capacity compared to the insoluble-bound phenolic compounds and non-defatted flaxseed has been shown to have more

(58 %) soluble free phenolic acids than insoluble-bound phenolic compounds (7-21 %) (Alu'datt *et al.*, 2013). This means the non-defatted flaxseed should have a higher antioxidant effect, which is consistent with the current results, as the flaxseed muffins had a significantly higher TPC and antioxidant capacity.

A study by Sęczyk *et al.* (2017) added flaxseed hull powder to bread as a flour replacement (1-5 %) and the results of the study found that adding flaxseed significantly ($p < 0.05$) enhanced the amount of polyphenols (12-93 %) compared to the control and the results for TPC were 0.47-0.81 mg GAE/g for bread with the different percentages of flaxseed compared to 0.42 mg GAE/g for the control bread. These results were in line with the current study as the results were 260, 414.4, 518.8 µg GAE/g for the control, whole and ground flaxseed muffins, respectively. In this study the ground flaxseed muffins were significantly higher in both total phenolic content and antioxidant capacity compared to the whole flaxseed muffins and this can be explained by the whole flaxseed still having the hull, which can affect nutrient availability.

Removing the hard coat by using different methods such as crushing, dehulling, grinding etc. can improve the nutrient availability and this leads to a higher TPC and antioxidant capacity seen for the ground flaxseed (Kajla, Sharma and Sood, 2014). The difference between these results is explained by use of flaxseed with hulls compared to dehulled flaxseed in different forms (whole and ground), as well as differences in the amount of flaxseed added as there was a higher percentage in this study (48 %) compared to the 5 % which was the highest used by Sęczyk *et al.* (2017).

Further differences could be due to the different products used (muffins vs. breads), and the two studies expressed their results in different units (μg GAE/g and mg GAE/g). The sample preparation methods were also different. Sęczyk *et al.* (2017) used a sliced and lyophilized bread sample using a freeze drier to produce a powder before extraction with 7.5 ml of a methanol: acetone: water mix with same proportion from each, while in the current study all the muffin samples were crushed and extracted using 70 % acetone.

The results for antioxidant capacity in this study were consistent with Kaur and Kaur (2018) who found that adding flaxseed flour from different Indian cultivars improved the antioxidant capacity compared to the control muffins, with a 13.37 % increase in 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) scavenging activity. In the current study, the results for the flaxseed muffins were significantly higher than the control muffins even when measured using FRAP assay.

Sęczyk *et al.* (2017) also found similar results for the antioxidant capacity using FRAP assay. They found that adding 1-5 % flaxseed hull powder increased the amount of antioxidants by 18-220 % compared to the control bread, while in the current study the increase was much higher 921-1782 % for whole and ground flaxseed compared to the control muffins. The higher amount of antioxidants could be due to the higher amount of flaxseed added to the muffins in the current study (48 %) compared to the amount of flaxseed flour (5 %) in the study by Sęczyk *et al.* (2017). This difference may also be due the use of hulled flaxseed in Sęczyk *et al.* (2017) but non-hulled flaxseed in the current study.

The results from Kaur, Singh and Kaur *et al.* (2017) showed the same trend; they found that increasing the concentration of flaxseed in cookies increased the antioxidant capacity measured by DPPH scavenging activity. The highest antioxidant capacity was 13.36 % in the flaxseed cookies compared to the control cookies. The differences between these results and the current study may be due to the different analysis methods used (FRAP vs. DPPH) and that may have led to different have different results (Prior *et al* 2005) as well as the different bakery products (cookies vs. muffins).

There are different factors that affect the concentration of antioxidants and TPC, which can cause different results in the literature. These factors include the flaxseed cultivars, regional and environmental conditions, the different extraction solvents used and the sample properties (whole flaxseed or defatted matrix) (Waszkowiak *et al.*, 2015). In this study, whole and ground flaxseed (dehulled or full fat) was used and used action as solvent.

Determining the glycaemic impact of a food depends on the rate of starch digestion due to its ability to control the level of glucose released into the gut due to the different the blood (Jenkins *et al.*, 1982). This process can result in a reduction in the high spike in the glucose level 30-45 minutes post meals, which can be more important than lowering the overall blood glucose (Ceriello *et al.*, 2008). SDS has been shown to cause a slower energy release than RDS and that results in a lower GR when measured in *in vivo* studies (Mishra and Monoro, 2009).

The results of RDS and SDS in the current study showed the addition of ground flaxseed significantly ($p < 0.05$) lowered the RDS compared to the control muffins,

which disagreed with the work of Sęczyk *et al.* (2017), as they found that bread enriched with flaxseed did not significantly lower the RDS when compared to the control bread. However, the results for SDS in both studies showed that there were no significant effects from flaxseed. There were differences in the study design, such as using bread or muffins, differences in flaxseed addition with 1-5 % in Sęczyk *et al.* (2017) compared with to 48 % in the current study and use of different forms of flaxseed (flaxseed hulls rather than non-hulled ground and whole flaxseed), which can help to explain the different results.

The explanation for lower RDS results for both flaxseed muffins compared to the control can be referred to the chemical composition of the muffins (Table 2.2 and Appendix 1.b), especially the amount of starch and CHO. The starch content was 20.4, 11.1 and 13.3 g/100g for the control, whole and ground muffins respectively. With regard to CHO, this was 40.9, 30.4 and 30.8 g/100 g for the control, whole and ground muffins respectively. The higher amount of CHO in the control muffins is due to the ingredients in the muffins (Table 2.1), especially the flour, which contained a higher amount of available CHO compared to the flaxseed which was used to replace it. However, the banana and sugar, which also contain CHO, were at the same amounts in all muffins.

To understand the starch digestion process which dependent on physical state and structural characteristics (molecular structure, starch granule morphology, degree of branching, amylose/amylopectin ratio). These two factors are influenced by the processing conditions used (e.g. hydrothermal processing) and the other components in the food matrix (e.g. polysaccharides, proteins ,lipids and dietary fibre) (Singh ,Dartois and Kaur, 2010). Protein in the food matrix may reduce the starch digestion

rate; the interaction of proteins and starches significantly affected their digestibility. For example, even small amounts of protein have been shown to alter the functional properties and starch digestibility of cereals and other food products (Singh Dartois, and Kaur 2010). Key proteins that can influence starch digestibility include glutenins, globulins and albumin, as they can help in glue starch granules into a matrix surrounded by proteins that can act as a barrier (Hamaker and Bugusu, 2003). This can be seen in the current results as flaxseed muffins contained 6.4 g/100g and 7.3 g/100g protein for the whole and ground flaxseed muffins respectively (Table 2.2) compared to 5.5 g/100g for the control.

Starch can also form complexes with lipids, which can cause significant behavioural changes in the starch, including delayed retrogradation, increases in gelatinization temperature, decreased solubility and resistance to being digested by enzymes. Lauric acid can bind to the glucose chain of amylose in the ratio of one molecule per w20 glucose units, but under the same conditions a very low amount of lauric acid bonds will bind to amylopectin and other branched glucans (Crowe, Seligman and Copeland, 2000). This can be seen in the current results as the flaxseed muffins contained a higher amount of fat with 15 g/100g and 14.9 g/100g in the whole and ground flaxseed muffins, respectively (Table 2.2) compared to 8.5g/100g for the control.

The role of fibre can be seen in reducing the sugar released from the starch in the food and this would reduce the RDS. Dietary fibre that can be dissolved in water alters the speed of starch digestion and degradation by changing the consistency, composition and thickness of food (Brennan, 2005). This can be seen in the current results as the flaxseed muffins contained a higher amount of fibre with 5.6 g/100g and 4.9 g/100g in

the whole and ground flaxseed muffins, respectively (Table 2.2) compared to 1.6g/100g for the control.

Studies have shown that starch bio-accessibility in a food can be affected by the incorporation of functional components such as phenolic-rich ingredients (Świeca *et al.*, 2014; Goh *et al.*, 2015; Sęczyk *et al.*, 2017;). Rendón-Villalobos *et al.* (2009) investigated this effect using tortillas fortified with flaxseed flour (10, 15 and 20 % replacement of corn flour) and the results suggested the low starch content in flaxseed caused a dilution of the starch in the tortilla, with an increase in RS due to the flaxseed flour (Rendón-Villalobos *et al.*, 2009).

It is possible for TPC in muffins (control, ground and whole flaxseed) to be derived from the several ingredients in the recipe, and not exclusively from the flaxseed (Table 2.1). It has been demonstrated, for example by Kandaswamy and Aradhya (2014), that banana is a rich source of several polyphenolic compounds such as gallic acid, catechin, epicatechin, tannins, and anthocyanins all of which have antioxidant activities. Aziz *et al.* (2011) conducted an analysis into banana for functional and chemical properties. Their analysis revealed the following contents: antioxidant capacity, cellulose, flavonoids, free-radical scavenging, insoluble dietary fibre, lignin, polyphenols, and total dietary fibre.

Cinnamon (*Cinnamomum zeylanicum*) is another ingredient which contains several antioxidative components (Muchuweti *et al.*, 2007). Among a number of herbs and spices (marjoram, mint, oregano, parsley, rosemary, sage and sweet basil), cinnamon has been shown to have the highest concentration of polyphenolic compounds at 13.7 mg GAE/g (Muchuweti *et al.*, 2007). Another rich source of phenolic compounds is

the wheat-grain flour of the muffin. In wheat, the highest phenolic compounds are flavonoids, phenolic acids, and lignans which are found in the outer grain layer (Žilić, 2016). The wheat fraction known as the aleurone layer has the greatest antioxidant activity, with bran having the second greatest. Žilić *et al.* (2012) found that debranned flour from bread and durum wheat contained 3.7 and 2.4 times lower antioxidant capacity than the respective bran fractions, however, the flour still contained a high level of antioxidants.

TPC from all of the aforementioned ingredients, as well as from the flaxseed, can have an impact on starch digestibility. TPC can affect the starch digestibility, as the polyphenols can inhibit the starch breakdown to glucose by affecting the digestive enzymes such as α -amylase and α -glucosidase (McDougall and Stewart, 2005). This may result in a reduction of the sugar released from the starch in the food and this would reduce the RDS. This agrees with the results of this study as the flaxseed muffins, especially the ground flaxseed, had a higher TPC (Figure 2.2) with 518.8 μg GAE/g compared to 260 μg GAE/g for the control muffins and also a lower RDS (Table 2.3) when compared to the control muffins with results of 42.3mg/g sample compared to 291.5 mg/g sample for the control muffins.

This impact may also be due to the nutritional composition of the flaxseed muffins which contain a high amount of protein, fat and fibre (see Table 2.2). Protein may reduce the starch digestion rate; the interaction of proteins and starches significantly affected their digestibility by altering the functional properties of the starch as it can help in glue starch granules into a matrix surrounded by proteins that can act as a barrier for the digestive enzymes (Hamaker and Bugusu, 2003 ; Singh, Dartois, and Kaur 2010). Fat which can cause significant behavioural changes in the starch,

including delayed retrogradation, increases in gelatinization temperature, decreased solubility and resistance to being digested by enzymes (Crowe, Seligman and Copeland, 2000). Fibre may reduce the sugar released from the starch and this would reduce the RDS. Dietary fibre that can be dissolved in water alters the speed of starch digestion by changing the consistency, composition and thickness of food (Brennan, 2005). Such improvement in the flaxseed muffins can result from the interaction between the polyphenols, protein, fat and fibre which leads to decreased solubility and increased resistance to being digested by enzymes which in turn reduces the rate of starch digestion (Crowe, Seligman and Copeland, 2000; Brennan, 2005; Singh Dattois, and Kaur 2010).

In order to investigate the impact of polyphenols on starch digestion alone, it has been suggested that it would be helpful to study the impact on starch digestion of added flaxseed polyphenols. This is appropriate because numerous studies have demonstrated that pure polyphenols and dietary phenolic extracts have the potential to delay starch digestion *in vitro*, and the delaying impact is dependent on the molecular structure and the phenolic composition (Sun and Mia, 2019).

Furthermore, it has been reported that *in vitro* inhibitory activities are associated with polyphenolic food extracts which include: berries (blackcurrants, blueberries, raspberries and strawberries), vegetables (beans, eggplant, maize and pumpkin), black tea, green tea, and red wine (Hanhineva *et al.*, 2010). An investigation into the extracts of a number of cultures of raspberries and their impact on starch-digesting enzymes *in vitro* was conducted by Zhang *et al.* (2010). Their investigation discovered that raspberry extracts inhibited α -glucosidase, but not α -amylase.

According to McDougall and Stewart (2005), α -glucosidase activity was inhibited, especially by the polyphenols diacylated and acylated anthocyanins.

All the above research supports the conclusion that polyphenols, especially a high source of polyphenols, have an effect on starch digestion. Future research should examine if adding polyphenols alone to the muffins rather than the whole flaxseed is as effective in lowering sugar release. To the best of our knowledge no studies have been done on the effect of added derived lignan to bakery products on sugar release.

Coe, Fraser and Ryan (2013) compared different of tea extracts (green, white and black) to a control bread and their results are similar to the current study in regards to the SDS and RDS that breads with added green tea extraction resulted significantly lower RDS compared to the control bread and insignificantly for the SDS results. There could be potential to look at something similar with extracts from flaxseeds.

Another factor that affects the starch digestibility is the processing methods of the cereals. Studies have shown that there can be an increase in digestibility of cereals due to the use of processing methods including soaking, dehulling and germination. This is due to the reduction in polyphenols, phytic acid and tannins, which normally decrease starch digestibility by inhibiting α -amylase activity. The loss of phytic acid and tannins can potentially produce large spaces in the matrix, which allows for increased enzymatic activity and improved starch digestibility (Rehman and Shah, 2005). Within this study, this effect can help to explain the lower (but not significantly different) RSD observed for the ground flaxseed muffins compared to the whole flaxseed muffins, which suggests the grinding of the flaxseed increased

starch digestibility. The effect on starch digestibility was not large as the whole flaxseed still lowered the amount of RSD.

2.5 Summary

This study has provided important information on the effect of adding flaxseed in different forms (whole and ground) to a standard muffin on the TPC and antioxidants capacity. The results show that ground flaxseed produced significantly higher levels for both TPC and antioxidant capacity and both flaxseed forms reduced the RDS in the muffins and this may lead to lower blood glucose levels. All the data from the *in vitro* study supported the decision to conduct *in vivo* studies on the effect of adding flaxseed in muffins on the blood glucose levels in human participants. This study provided new data on the *in vitro* digestion of flaxseed muffins with regards to starch digestibility and the results suggested there was a need to conduct a study to determine the GI values of the flaxseed muffins. This was the aim of the next chapter of this thesis.

Chapter 3 : Determination of the Glycaemic Index (GI) of different types of muffins (controls, whole and ground flaxseed)

3.1 Justification and aims

Jenkins *et al.* (1981) was the first to introduce the concept of GI, which was developed to help rank dietary carbohydrates and can represent the quality of the carbohydrate within a food. The ranking is split into three groups based on the overall effect of the CHO on postprandial blood glucose concentration and is measured relative to a reference CHO, which is generally pure glucose or white bread (Brouns *et al.*, 2005). High GI foods are those that measure ≥ 70 on the glucose scale and generally contain CHO that are easily absorbed, digested and metabolised; intermediate GI foods measure between 56 and 69 on the glucose scale and low-GI foods measure ≤ 55 and contain CHO that are slowly digestible, which means they produce a reduced postprandial glucose response (Augustin *et al.*, 2015).

There are benefits of following a diet containing low GI foods; these types of diet can be used to help manage obesity by influencing the GR, help individuals with diabetes manage their health and control their blood glucose and even help with weight loss in healthy individuals (Schwingshackl and Hoffmann, 2013). Low-GI foods can be beneficial for satiety, as they produce a higher satiety than high-GI foods by increasing fat oxidation instead of carbohydrate oxidation. These abilities arise from the fact that low-GI foods are digested at a slower rate and absorbed slower, which has an effect on postprandial hyperinsulinemia and glycemia (Jenkins *et al.*, 1994; Ludwig, 2000).

Bakery products such as bread and muffins are generally classed as medium-to-high GI foods due to the fact they are cereal products that contain high levels of white flour

and sugar; therefore being able to reduce the GI of these products would therefore be beneficial as they are frequently consumed (Ferrer-Mairal *et al.*, 2011). To reduce the GI, both the baking processes and raw materials used can be altered to influence GR (Fardet *et al.*, 2006). For example, inclusion of other types of flours or grains into wheat flour in bakery products can reduce the GI as this increases the amount of fibre in the product, which slows down digestion (Åkerberg, Liljeberg and Björck, 1998).

It has been shown that fibre can reduce GI values and this is especially true for soluble fibres such as mucilage, pectin and gums (Wolever *et al.*, 1990b; Vinoy *et al.*, 2017). Studies have shown that high fibre content can reduce the GI of food by significantly reducing blood glucose levels after eating by slowing down sugar released (Thakur *et al.*, 2009; Kapoor *et al.*, 2011). A good example of a seed that contains high fibre content is flaxseed, as it contains 28 % dietary fibre with the main source of soluble fibre being mucilage gum. This has led to the classification of products containing high levels of flaxseed as low GI in the international tables by Atkinson *et al.* (2008) such as Bürgen Bread Soy & Linseed from Tip Top Bakeries, Australia with GI of 36, Vogel' s® Ultra Bran® Soy & Linseed extruded wheat bran cereal from Australia with GI of 41.

As flaxseed has an excellent nutrient profile and health-promoting properties it is becoming more popular in the human diet, especially when it is incorporated into other products or used in functional foods. The components of flaxseed include omega-3 fatty acids, fibre and lignans, which can produce health benefits. Flaxseed is also a good source of phenolic compounds, soluble fibres and high quality protein, which can have health benefits (Oomah, 2001).

The aim of this study was to compare the GI values of different types of muffins (control, control containing no flaxseeds and two muffins with flaxseeds incorporated in different forms - whole and ground) as there is an interest in incorporating flaxseed into bakery products and there is a need to investigate whether flaxseed would improve the GI when added in different forms. To the best of our knowledge, there are no data comparing the GI value of flaxseed in different forms (whole and ground) incorporated into bakery products such as muffins.

3.2 Methods

3.2.1 Participants

Healthy male and female adults aged between 18-60 years were recruited in this study at Oxford Brookes University through advertisements and personal communications between October 2016 to January 2017. Ethical approval for this study was obtained from the University Research Ethics Committee (UREC) at Oxford Brookes University, under the Oxford Brookes Centre for Nutrition and Health's programme application: Glycaemic response (GR) and glycaemic index (GI) testing of foods (UREC Registration No: 140806).

Any participants that were interested in the study were sent Participants Information Sheet (PIS) by email (Appendix 2a) and were invited to attend the Oxford Brookes Centre for Nutrition and Health at least 24 hours later to discuss and queries and sign the consent form. At this point the participants were asked to complete a screening questionnaire to ensure they were eligible to take part in the study.

The inclusion criteria for participants were:

- Aged 18-60 years.
- $\text{BMI} \leq 30\text{kg/m}^2$.
- Fasting blood glucose < 6.1 mmol/l.
- Not pregnant or lactating.
- No known diabetes or impaired glucose tolerance.
- No medical condition(s) or medication(s) known to affect glucose regulation or appetite and/or which influence digestion and absorption of nutrients.
- No major medical or surgical event requiring hospitalisation within the preceding three months.
- No food allergy to muffin ingredients (eggs, milk, wheat flour and flaxseed).

If a participant presented with a disease, or took medication known to interfere with glucose metabolism and/or insulin signalling, they were excluded from the study.

3.2.2 Study design

The study was a randomised, cross-over repeated-measures design. Randomisation of participant order of tested food was carried out using a random number generator (<https://www.randomizer.org>). The study involved seven visits: an initial screening visit and six subsequent visits to test the different muffins or the standard glucose.

The interested participants were provided with instructions about preparation for the screening visit before coming for the screening session; Participants needed to avoid the consumption of caffeine, alcohol and nicotine and to avoid unusual, strenuous exercise prior to the test sessions. The consumption of alcohol, especially in a fasting

state, can greatly affect glucose homeostasis (Shelmet *et al.*, 1988; Siler *et al.*, 1998). Another potential confounding factor is smoking cigarettes, as it can cause acute insulin resistance (Attvall *et al.*, 1993; Frati, Iniestraand and Ariza, 1996). Further changes in glucose homeostasis can be due to acute physical activity, as this has been shown to increase glucose tolerance and insulin sensitivity for up to 2 days by improving muscle glucose uptake (Campbell, Glowczewski and Wolever, 2003). Another factor that needs to be taken into account when investigating GR in humans is caffeine intake, as caffeine can acutely decrease insulin sensitivity (Keijzers *et al.*, 2002). Participants were advised to wear loose, comfortable clothing, with outer garments which can easily be removed for body measurements.

During the screening visit, participants were provided with the PIS to keep and they could ask any questions regarding taking part in the study. If they agreed to take part in the study, they were then asked to sign a consent form (Appendix 2b). Participants were asked to complete a health questionnaire (developed by the Oxford Brookes Centre for Nutrition and Health at Oxford Brookes University), which included questions regarding to food allergies/intolerances, metabolic diseases, smoking habits, physical activity, medical conditions and medication (Appendix 2c). After completing the questionnaire, the following anthropometric measurements were taken: height using a free-standing stadiometer (SECA, Germany), body weight, body fat percent and fat-free mass using the Tanita body composition analyser (BC-418, Tanita UK Ltd, Middlesex, UK). Height was recorded to the nearest 0.1 cm, whereas body weight was recorded to the nearest 0.1 kg. If the participants were eligible after taking the anthropometric measurements and did not have any medical conditions or food allergies, then a fasting blood glucose measurement was taken after fasting overnight for 12 hours.

Capillary finger-prick blood samples ($5 \mu\text{l}$ of blood) were used for determining glucose levels using a HemoCue®201⁺ Glucose analyser to determine the baseline data for glucose and if the participant was eligible to participate in the study. Once screening had been completed, participants were booked in for the rest of the test sessions to test each of the muffins (control, whole flaxseed and ground flaxseed) one time and a standard food (oral glucose solution) three times according to guidelines ISO (2010). Muffins were compared with the standard food in equivalent available carbohydrate amounts of 50 g.

On the test day participants arrived at Oxford Brookes Centre for Nutrition and Health in the morning (7.00-9.30 am). Each test session lasted for two hours (Brouns *et al.*, 2005). Capillary finger-prick blood samples were taken at 0, 15, 30, 45, 60, 90 and 120 minutes post consumption to determine the blood glucose value during each test sessions (Appendix 2d). A one-day washout was needed between the test sessions to minimize the carry over effect on the participant.

3.2.3 Standard food

Most studies use glucose rather than white bread as a standard food to compare with the test food to determine GI values. In this study, glucose (dextrose monohydrate; Lloyds Pharmacy Limited, Coventry, UK) was used based on the recommendation of ISO (2010) to use glucose in preference to white bread, as it is easier to standardize the CHO source. There has been a great deal of discussion around the use of bread or glucose as a reference food, especially as bread is a commonly consumed food, which could mean that it would allow GI to be determined in a more physiological manner. However, there might be experimental variation due to differences between the

compositions of brands of white bread, which would make comparing the results between studies difficult. This variability has been investigated in a inter-laboratory study, which found that locally obtained bread samples were no more variable for their GI values than centrally provided (comparable) foods (Pieters and Jerling 2005). This result is in contrast to another study, which found that white bread from certain local areas (such as white bread French baguette) can have GIs that are different to a common white bread from other areas (Foster-Powell, Holt and Band-Miller, 2002). Overall, if white bread is used as the reference the GI values are typically about 1.4 times greater than when glucose is used as the reference (FAO, 1998). Generally, glucose is preferred as the source of CHO as it is more standardised, but glucose consumption as a reference can occasionally cause subjects to become nauseous after an overnight fast.

To prepare the glucose drink, 54.96 g of dextrose monohydrate was dissolved with 250 ml of water to provide 50 g of available CHO and the participants were required to consume it within 15 minutes.

3.2.4 Test foods

A standard recipe was used to prepare the different types of muffin (flaxseed and control) (Section 2.2.1)

To make the comparison between muffin types and the control (standard glucose), the muffins were served as 50 g of available CHO equivalents (Table 3.2). The quantity of each muffin that equalled 50 g of available CHO was calculated using the following equation:

$$Muffin\ weight = \frac{(50 \times 100)}{\text{available carbohydrate in 100 g of muffin}}$$

(FAO/WHO, 1998; Wolever *et al.*, 2003).

The amount of the flaxseed which was added to the muffins in order to provide 50 g available carbohydrate was 21g for both flaxseed types.

Table 3.1. Available carbohydrate, sugar and starch content in each serving for each muffin type

Muffin	Serving size	Available carbohydrate	Total sugar	Starch
	g	g	g	g
Control	122.2	49.9	22.8	24.9
Whole	164.5	50.1	30.4	18.3
Ground	162.3	50.3	29.7	21.7

3.2.5 Blood collection and analysis

The methods of the ISO guidelines (2010) were applied to determine the GI. A single-use lancing system was used to take capillary finger-prick blood samples (Unistick 3, Owen Munford, UK). The participants needed to have warm hands and be relaxed. To develop a drop of blood the finger was massaged then squeezed to produce the drop, which was used in the test.

The use of capillary blood sampling for GI tests is considered to be the gold standard, as it allows detection of changes in the glucose level quickly and can produce more acute results than venous blood sampling (Brouns *et al.*, 2005). Capillary blood samples were taken during fasting, at -5 and 0 minutes and the mean was calculated and used as the baseline for glucose level. Within 15 minutes of recording the baseline measurement, participants consumed either the relevant muffins (served with

250 ml water) or the standard glucose (50 g in 250 ml water). For a 2-hour test, capillary blood samples were taken at 15, 30, 45, 60, 90 and 120 minutes after the start of consumption of the product under investigation.

A HemoCue®201⁺ glucose analyser (Glucose 201⁺: Hemocue AB, Sweden) ,which is an automatic analyser that provides a glucose reading within 40-240 seconds, was used to measure blood glucose. This analyser uses a photometric system based on a modified glucose dehydrogenase method. To analyse glucose in the blood samples, a disposal plastic microcuvette containing all the reagents on its wall used. As the blood is dropped into the cavity, a reaction occurs between the reagents and the blood (HemoCue Hb 201⁺, Operating manual), which then allows the blood glucose reading to be taken. To perform each measurement only 5 μ l of blood was required.

Studies have assessed the reliability of the blood glucose measurements using HemoCue analyser, such as the work by Voss and Cembrowski (1993) and Stork *et al.* (2005), which investigated HemoCue Beta-glucose analyzer (HemoCue) accuracy and how the results from whole blood correlated with the Yellow Springs Instrument (YSI 2300 STAT; YSI) glucose oxidase analyser results in plasma. The studies found that the HemoCue B-Glucose analyzer was a simple, reliable and rapid detection method for whole-blood glucose level determination.

Prior to each test, a quality control test was carried out to ensure that the results were within the range of the analyser. Based on the manufacturer's recommendations, the Gluco Trol-NG Level 2 (Eurotrol, The Netherlands) was used for the quality control check (NHS PASA, 2006).

3.2.6 Calculation of GI

For each muffin type and the standard glucose, the iAUC using the trapezoidal rule (without the area beneath the baseline) was calculated using ISO guidelines (2010). To reduce intra-individual variability, each participant consumed the standard food test on three separate occasions and the GI values were calculated from the mean of these values in accordance with ISO guidelines (2010). The coefficient of variation (CV) was calculated for the 3 tests; if it was greater than 30 % then repeat tests were carried out until the CV was under 30 %. Within GI studies it is important to consider differences due to within-subject variation, as this can be the largest source of GI value variation. This is due to the GI being a ratio of two independently variable AUC values for the glycaemic response, which means the variation of GI values can be greater than the within-subject variation for the GR. Increases of within-individual variation for the GR can skew the GI value distribution, which can increase the mean (Wolever *et al.*, 1990b; Brouns *et al.*, 2005).

The iAUC for each muffin type eaten by each participant was reported as a percentage of the mean iAUC of the reference food for the same participant, then for each food the mean GI was calculated for the whole group. The GI of each muffin was calculated using the formula:

$$GI = \frac{\text{Plasma glucose iAUC value for test food}}{\text{Average iAUC value for equal carbohydrate portion of the reference food}} \times 100$$

(Jenkins *et al.*, 1981).

3.2.7 Statistical analysis

Based on the ISO guidelines (2010), the number of participants needed was 10 participants, to give the study a higher degree of power of accuracy within the test. Statistical analysis was carried out using the Statistical Product and Service Solutions Software (SPSS) version 25.0 (Chicago, IL, USA). Data are presented as mean, SD and SEM. Prior to statistical analysis; the normality of the data was measured using the Shapiro–Wilks statistical test. The levels of inter- and intra-individual variation were measured using CV % analysing the three glucose (standard) tests. Repeated measures ANOVA using Bonferroni for multiple comparisons (for parametric data) and Friedman and Wilcoxon tests (for non-parametric data) were used to compare the iAUC and GI values of the three types of muffin. Statistical significance was set at ($p = 0.05$).

A Bonferroni correction was used to correct for multiple comparisons of the results of the change from the baseline for blood glucose, by dividing the p value of 0.005 by 6 (number of the comparisons: $p < 0.008$ for those results. A Bonferroni correction can be defined as an amendment of the p values when many independent or dependent statistical tests are being simultaneously undertaken on one data set. A Bonferroni correction is conducted by dividing the critical p value by the number of comparisons being applied. The reason for the application of the Bonferroni correction is to reduce the possibility of receiving false positive results (type I errors) during the application of multiple pair-wise tests on one data set (Matthew and Napierala, 2012).

3.3 Results

3.3.1 Participant characteristics

Eleven participants (seven males, four females) were recruited to the study. One male participant was excluded from the study as the CV for his standard glucose was >40 % following ISO guidelines (2010). Thus, the number of the participants who successfully completed all session in this study was ten (six males, four females). The baseline characteristics of the participants are shown in (Table 3.3).

Table 3.2. Baseline characteristics of ten participants (4 female, 6 male) (mean $\pm$ SD)

	All (<i>n</i> 10)
Age (y)	29.5 $\pm$ 8.6
Height (m)	1.7 $\pm$ 0.1
Weight (kg)	68.1 $\pm$ 10.2
BMI (kg/m ²)	23.8 $\pm$ 2.3
Body fat %	21.3 $\pm$ 5.9
Fasting blood glucose (mmol/l)	4.9 $\pm$ 0.1

3.3.2 Blood glucose values

The mean CV for the blood glucose iAUC after 120 min within the three standard tests was 16.6 %, which is consistent with the recommendation from Wolever (2006) for the CV for blood glucose for healthy subjects to range between 12-39 %. The mean CV for the iAUC after 120 min in the muffins was 63.7 % for the control, 49.3 % for whole flaxseed muffins and 49.7 % for the ground flaxseed muffins.

The results for the blood glucose levels over time (Table 3.4) showed that for all muffins (control, whole and ground flaxseed muffins) all blood glucose levels

returned to the baseline level after 2 hours and this was the same for the standard glucose.

Table 3.3. Blood glucose levels (mmol/l) after consumption of the standard glucose and the three different muffin type (mean $\pm$ SD).

Time (min)	Glucose*	CM	WFM	GFM
0	4.9 $\pm$ 0.5	4.9 $\pm$ 0.5	4.9 $\pm$ 0.5	4.9 $\pm$ 0.5
15	6.6 $\pm$ 0.4	5.7 $\pm$ 0.6	5.3 $\pm$ 0.4	5.6 $\pm$ 0.5
30	7.5 $\pm$ 0.5	6.8 $\pm$ 0.4	6.8 $\pm$ 0.5	6.3 $\pm$ 0.8
45	7.0 $\pm$ 0.5	5.9 $\pm$ 0.5	6.2 $\pm$ 0.9	5.8 $\pm$ 0.5
60	6.3 $\pm$ 0.3	5.3 $\pm$ 0.5	5.4 $\pm$ 0.6	5.2 $\pm$ 0.6
90	5.5 $\pm$ 0.3	5.0 $\pm$ 0.5	5.0 $\pm$ 0.5	5.1 $\pm$ 0.4
120	4.4 $\pm$ 0.4	5.0 $\pm$ 0.6	4.9 $\pm$ 0.4	5.0 $\pm$ 0.5

CM = control muffin; WFM = whole flaxseed muffin; GFM = ground flaxseed muffin

*Mean of three standard glucose tests

3.3.3 Blood glucose iAUC

The results for iAUC for the blood glucose response to the standard glucose and to the three types of muffins (control, whole and ground flaxseed) (Table 3.5) showed that there were a significant differences between the glucose standard and the control muffins, with a reduction in the iAUC (mmol.min/l) after 60 and 90 min ($p < 0.05$). Likewise, there were significant differences between the glucose and the whole flaxseed muffins and between the standard glucose and the ground flaxseed muffins, with a reduction in the iAUC (mmol.min/l) for all time points ($p < 0.05$).

Table 3.4. Blood glucose iAUC (mmol.min/l), for each muffin type compared to the standard glucose (mean $\pm$ SD).

	Glucose*	CM	WFM	GFM	<i>p</i> value
iAUC 60	106.4 $\pm$ 30.8	64.7 $\pm$ 26.5 ^a	59.8 $\pm$ 22.6 ^a	47.4 $\pm$ 20.9 ^{ac}	< 0.001
iAUC 90	138.4 $\pm$ 57.8	77.5 $\pm$ 44.2 ^a	72.1 $\pm$ 32.8 ^a	57.2 $\pm$ 26.4 ^a	< 0.001
iAUC 120	149.7 $\pm$ 69.6	87.5 $\pm$ 55.7	78.9 $\pm$ 39.6 ^a	63 $\pm$ 29.4 ^a	0.001

^a Significant differences ($p < 0.05$) compared to the standard glucose.

^c Significant differences ($p < 0.05$) compared to whole flaxseed muffin.

CM = control muffin; WFM = whole flaxseed muffin; GFM = ground flaxseed muffin

* Mean of three standard glucose tests.

The results for the post-hoc multiple pair-wise comparisons between the glucose and all muffin types for the iAUC (Table 3.6) at all time points (60, 90 and 120 min) showed significant differences between the standard glucose and all muffin types. At 60 min, there was a significant reduction in the iAUC for ground flaxseed muffins compared to whole flaxseed muffins ($p=0.035$). This was the only significant difference between the two types of flaxseed muffins. The rest of the iAUC did not show any significant differences between the muffin types.

Table 3.5. Post-hoc multiple pair-wise comparisons for blood glucose iAUC for each muffin type compared to the standard glucose (mean $\pm$ SD)

iAUC 60 (mmol.min/l)				
	Glucose	CM	WFM	GFM
Glucose		0.007	< 0.001	< 0.001
CM	0.007		1	0.218
WFM	< 0.001	1		0.035
GFM	< 0.001	0.218	0.035	
iAUC 90 (mmol.min/l)				
	Glucose	CM	WFM	GFM
Glucose		0.039	< 0.001	< 0.001
CM	0.039		1	0.675
WFM	< 0.001	1		0.123
GFM	< 0.001	0.675	0.123	
iAUC 120 (mmol.min/l)				
	Glucose	CM	WFM	GFM
Glucose		0.114	0.003	0.002
CM	0.114		1	0.743
WFM	0.003	1		0.383
GFM	0.002	0.743	0.383	

CM = control muffin; WFM = whole flaxseed muffin; GFM = ground flaxseed muffin.

The results of the change in glucose concentration (Figures 3.1-3.4) from baseline showed that there were differences between the muffin types (control, whole and ground flaxseed) and the standard glucose. The differences were significant ($p < 0.008$) for the time points 15, 30 and 45 and 60 minutes after consumption, with lower blood glucose levels comparing to the standard glucose level for all time points except for after 120 min where the glucose level was higher for the muffins than for the standard glucose. For 15 and 60 minutes the difference were between both flaxseed muffins (whole and ground) and the standard glucose. While on 30 minutes

the difference was between the standard glucose and the ground flaxseed. For the 45 minutes the difference was between the control muffins and ground flaxseed muffins compared to the standard glucose. These results are consistent with the results of the iAUC values (Table 3.3). Ground flaxseed was by far the lowest in that time point.

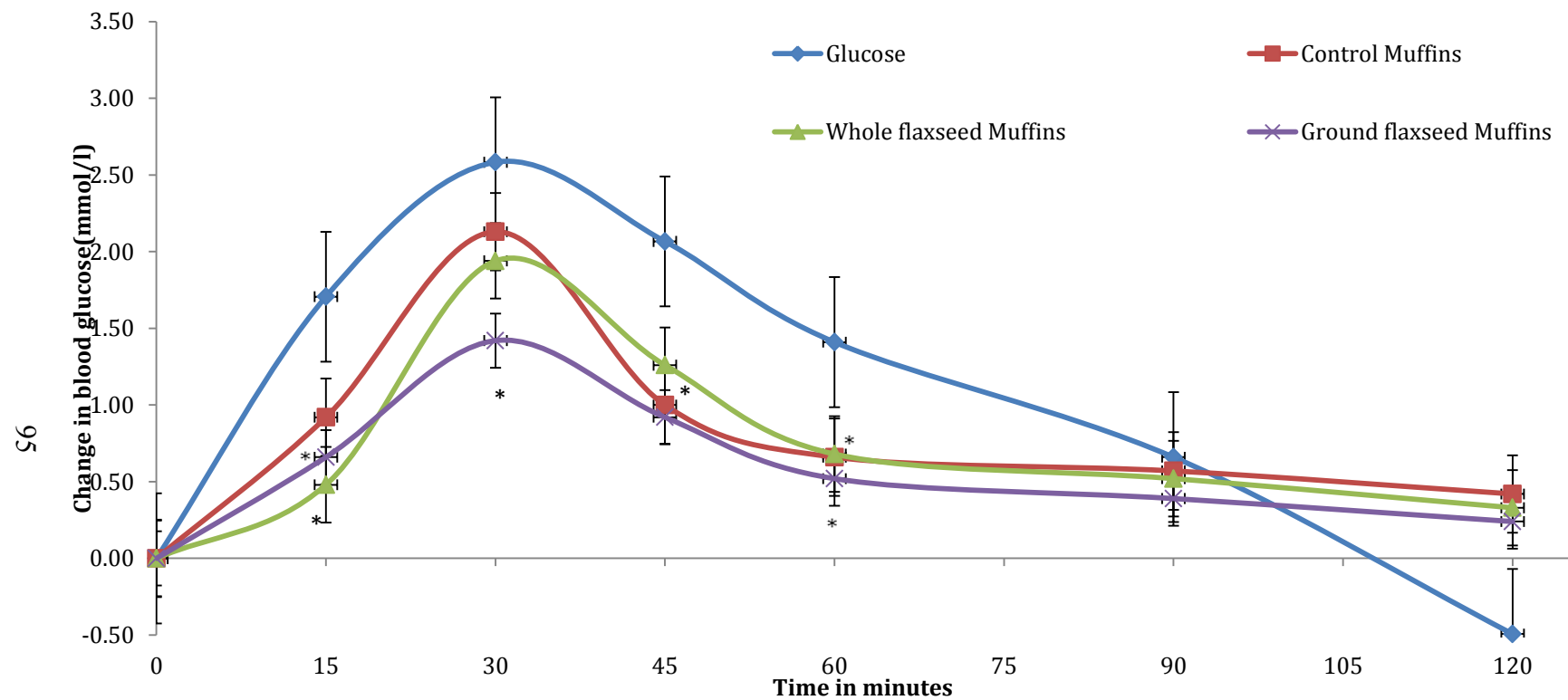


Figure 3.1. Change in the blood glucose concentration (mmol/l) over time for each muffin type compared to the standard glucose. Values mean difference $\pm$ SEM (n-10).

*Significant differences $p < 0.008$ (after Bonferroni correction) compared to the standard glucose.

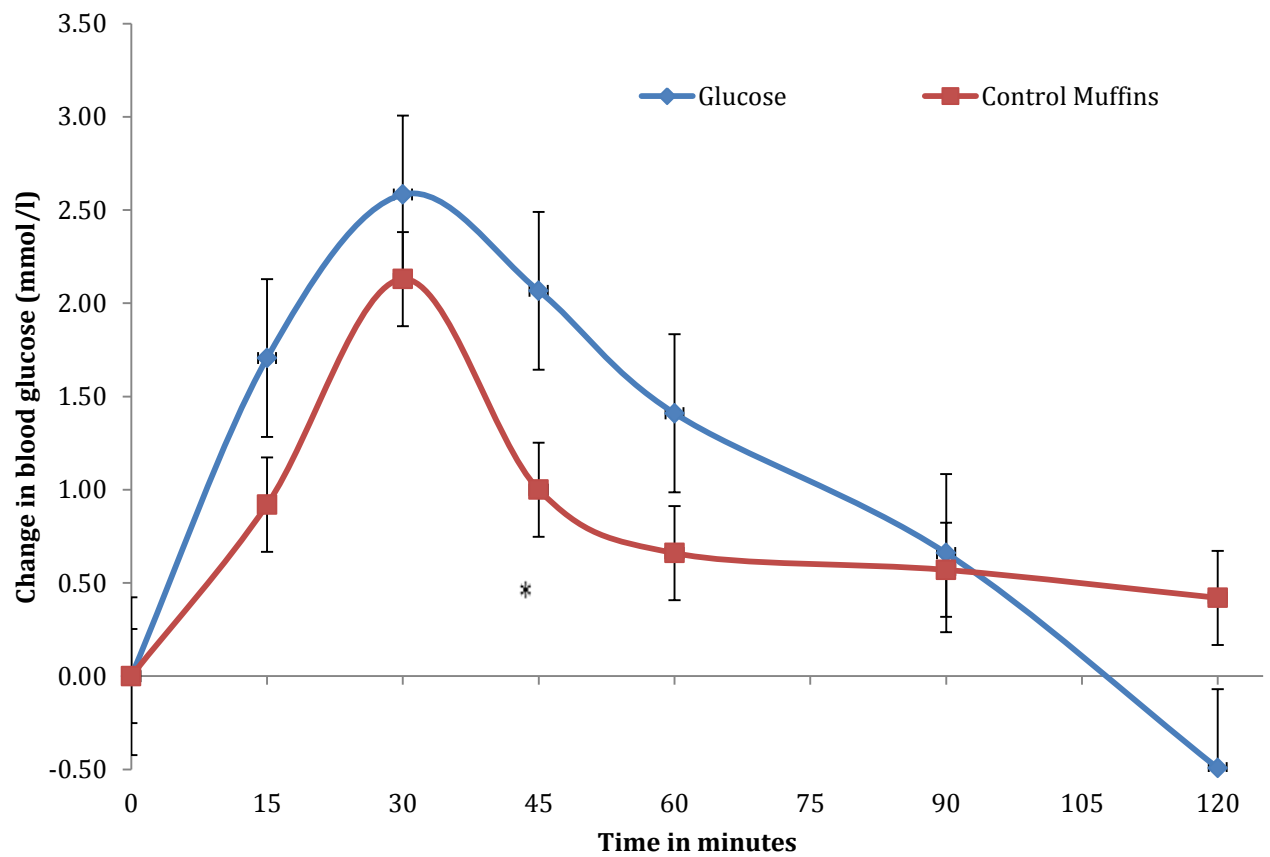


Figure 3.2. Change in the blood glucose concentration (mmol/l) over time for control muffin type compared to the standard glucose.
 Values mean $\pm$ SEM (n=10).
 *Significant differences $p < 0.008$ (after Bonferroni correction) compared to the standard glucose.

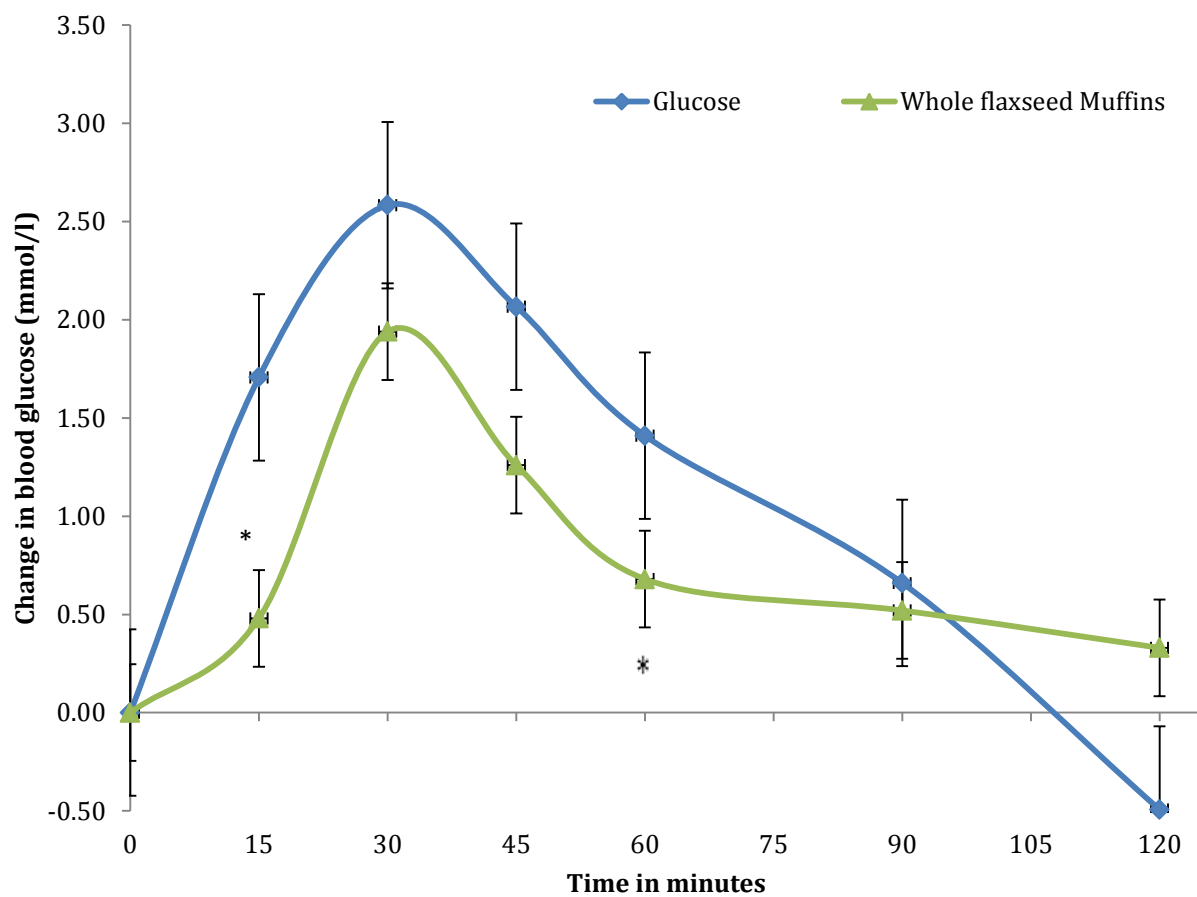


Figure 3.3. Change in blood glucose concentration (mmol/l) over time for whole flaxseed type compared to the standard glucose.

Values mean $\pm$ SEM (n=10).

*Significant differences $p < 0.008$ (after Bonferroni correction) compared to the standard glucose.

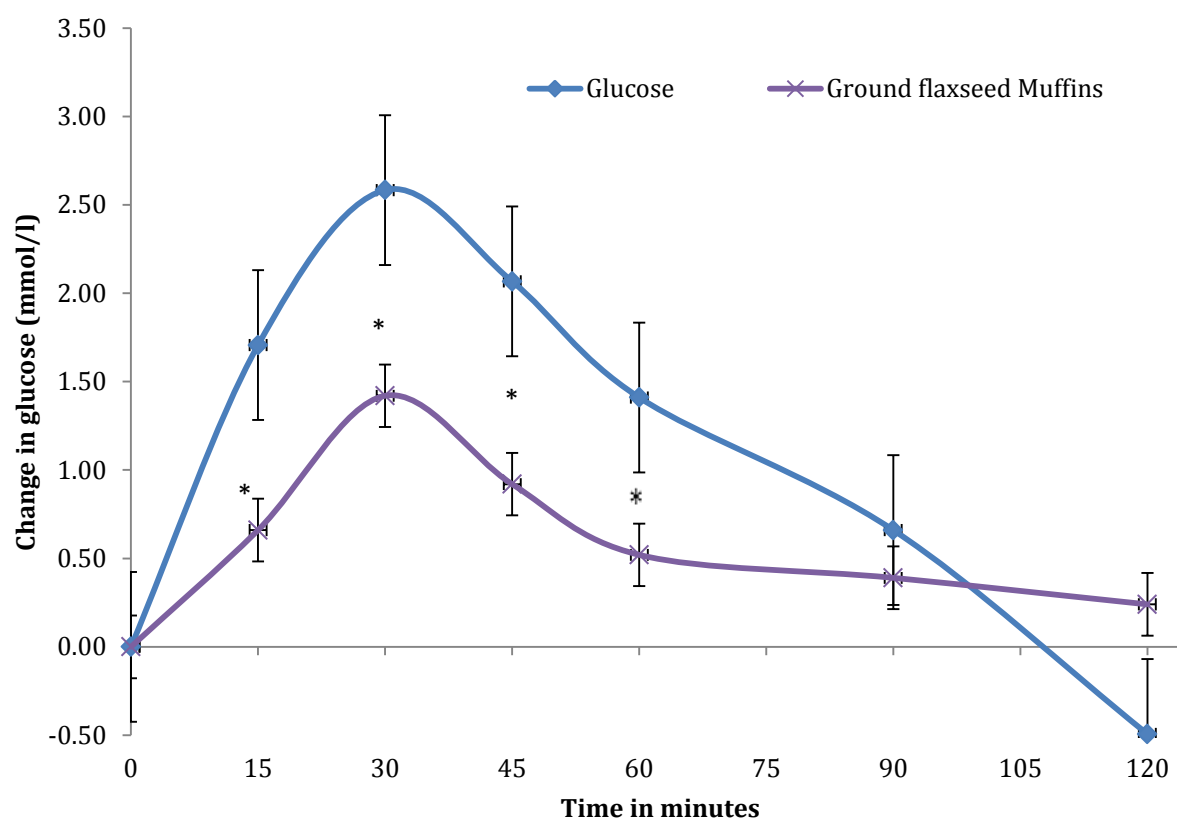


Figure 3.4. Change in glucose concentration (mmol/l) over time for ground flaxseed muffin type compared to the standard glucose.

Values mean $\pm$ SEM (n=10).

*Significant differences $p < 0.008$ (after Bonferroni correction) compared to the standard glucose.

3.3.4 Blood glucose peak values and time to peak

The results for the blood glucose peak values (Table 3.7) indicate that the values for all muffin types were lower than those for the standard glucose ($p > 0.05$). However, there were no statistically significant differences between the two-flaxseed muffin types. The results for time to peak showed no significant difference between all the muffin types and the standard glucose. The p values for the peak values were analysed by the post-hoc multiple pair-wise comparisons (Table 3.8). The results show that the

p values were 0.004 for control muffins, 0.003 for the whole flaxseed muffins and < 0.001 for the ground flaxseed muffins when compared to the standard glucose.

Table 3.6. Blood glucose peak values and time to peak for each muffin type compare to the standard glucose (mean $\pm$ SD).

	Glucose*	CM	WFM	GFM	<i>p</i> value
Peak (mmol/L)	7.7 $\pm$ 0.7	6.9 $\pm$ 0.4 ^a	6.9 $\pm$ 0.5 ^a	6.5 $\pm$ 0.6 ^a	< 0.001
Time to peak (min)	33 $\pm$ 13	30 $\pm$ 0	34 $\pm$ 6	32 $\pm$ 9	0.673

^a *Significant differences (*p* < 0.05) compared to the standard glucose.

CM = control muffin; WFM = whole flaxseed muffin; GFM = ground flaxseed muffin

* Mean of three standard glucose tests.

Table 3.7. Post-hoc multiple pair-wise comparisons for blood glucose peak values for each muffin type compared to the standard glucose (mean $\pm$ SD).

Peak (mmol/L)				
	Glucose	CM	WFM	GFM
Glucose		0.004	0.003	< 0.001
CM	0.004		1	0.097
WFM	0.003	1		0.051
GFM	< 0.001	0.097	0.051	

CM = control muffin; WFM = whole flaxseed muffin; GFM = ground flaxseed muffin

3.3.5 Glycaemic index

The results for the GI values for all muffins (Table 3.9) showed that adding flaxseed in either form (whole and ground) lowered the GI values, which meant they could be

classified as low GI, while the control muffin was medium in GI. The difference between the GI values was not significant ($p = 0.105$).

Table 3.8. GI value and classification for each muffin type

	CM	WFM	GFM	<i>p</i> value
GI value	64.3 ± 36.8	53.3 ± 13.2	42.2 ± 13.4	0.105
GI classification	Medium	Low	Low	

CM = control muffin; WFM = whole flaxseed muffin; GFM = ground flaxseed muffin

3.4 Discussion

The results of the change in glucose concentration (Figures 3.2-3.4) from baseline showed that the glycaemic response was highest in the control muffin compared to the two flaxseed muffins. This result was expected due to the composition of the different muffins (Table 3.1), in particular, the amount of starch, which was higher in the control muffin compared to the flaxseed muffins.

The results showed that the glucose values of all muffin types were significantly lower than that of standard glucose. There were also no significant differences between any of the muffin types except for iAUC 60 min post-consumption between ground and whole flaxseed. This result can be explained by the bioavailability of ALA and lignan, which are both responsible for glucose regulation.

For ALA, bioavailability depends on the type of flaxseed used; flaxseed oil has the highest bioavailability, followed by milled flaxseed and then whole flaxseed (Austria *et al.*, 2008). Research has shown that milling or crushing flaxseed can substantially improve entero lignan bioavailability (Kuijsten *et al.*, 2005) due to the improved accessibility of ground or crushed flaxseed for the colon bacteria, which can increase

digestion (Arterburn ,Hall and Oken, 2006;Rodriguez-Leyva *et al.*, 2010). The GI results indicate that, compared to the control muffins, flaxseed had the effect of lowering GI.

Regarding the blood glucose iAUC, Au *et al.* (2013) conducted a study to compare the effect of flaxseed fibre on other dietary fibres from different sources, such as soy and guar, in different forms, such as in a glucose solution or a as solid form. Dairy products such as pudding were then used to illustrate GR among healthy participants. Au *et al.*(2013) found that flaxseed lowered the iAUC compared to standard glucose, which is accordance with the results of the current study.

Providing further support for these results, Dah *et al.* (2005) conducted a study comparing the GI of bread enhanced by 15 g of flaxseed with that of white bread (both breads contain 50 g of available CHO). The results for iAUC at 120 minutes showed there was a significant difference between the flaxseed bread and white bread ($p = 0.015$) and that, consistent with the current study, there was a significant difference between the flaxseed muffins (whole and ground) compared to standard glucose ($p = 0.001$) in that flaxseed lowered the iAUC after 120 minutes.

The effect of flaxseed on a healthy individual's blood glucose level was also investigated by Cunnane *et al.* (1993), who incorporated flaxseed flour into bread (50 g of available CHO) and compared the results to white bread. Although conducted using bread rather than muffins, the results showed that, as in the current study, flaxseed lowered 60 min post consumption iAUC by 28 % compared to the control white bread (Cunnane *et al.*, 1993).

Research on blood glucose levels and diet has shown that flaxseed fibre can lower blood glucose levels. This important effect is explained by research into insoluble fibres, which has shown that these can slow the release of sugar into the blood, significantly reducing blood glucose levels after eating (Thakur *et al.*, 2009; Kapoor *et al.*, 2011).

Dietary fibre that can be dissolved in water alters the speed of starch digestion and degradation by changing the consistency, composition and thickness of food (Brennan, 2005). This effect is associated with the management of postprandial glucose levels. For instance, the rheological characteristics of viscous fibre means that it coagulates with the food and the products of human digestion, lessening concentrations of postprandial plasma glucose relative to viscosity levels in both diabetic and healthy people (Kim, Wong and Vuksan 1996). Viscous fibre enacts such an effect by slowing the speed at which glucose is digested and absorbed by the blood (Rayner *et al.*, 2001). Consequently, fibre in a more viscous mixture substantially lowers blood glucose levels (Jenkins *et al.*, 2008).

However, uncertainty remains regarding which digestive stages (gastric or small intestinal) are affected by viscosity and fibres and how these in turn impact on the hormones and enzymes created by the gut during the process of digestion which takes place within the gastrointestinal tract. The four pathways most commonly suggested are: delayed gastric emptying, modulated release of the gut hormones that regulate digestion and absorption, reduced digestive enzyme activity in the small intestine and delayed diffusion of hydrolysed starch fragments or glucose in the small intestine (Dikeman and Fahey, 2006; Grundy *et al.*, 2016).

In terms of the blood glucose peak value, the results of the current study are consistent with those of Au *et al.* (2013) who found that peak glucose was significantly lower in a pudding with flaxseed than standard glucose. These results are further supported by the work of Dah *et al.* (2005), who studied the effect of adding 15g flaxseed in bread on GR. The results identified a significant difference ($p < 0.05$) in peak glucose values, which were lower for the bread with flaxseed (6.6 ± 0.9 mmol/l) than the control bread (6.9 ± 0.7 mmol/l).

In the current study there were no significant differences in time to peak between muffin types (control, whole and ground flaxseed) and standard glucose. This agrees with the results from Au *et al.* (2013) who investigated the differences between glucose with flaxseed fibre and standard glucose and found there were no significant differences in time to peak.

The flaxseed muffins had a low GI of 53.3 and 42.2 for the whole and ground flaxseed, respectively. These results can be compared to those of international tables by Atkinson *et al.* (2008) such as rye soy with flaxseed bread (Country Life Bakery, Australia) with GI of 55 and Soy & Linseed bread (Con Agra Inc., USA) with GI of 50. This may be due to the nutritional value of flaxseed, especially the amount of protein, fat, and fibre it contains. Flaxseed contains a high amount of protein (20 %), fat (41 %) and dietary fibre (28 %) (Bernacchia *et al.*, 2014). Dietary macronutrients, such as protein and fat, can have an impact upon the postprandial blood glucose level. For instance, dietary fat slows glucose absorption, thereby delaying peak GR to the ingestion of food containing glucose (Collier and O'Dea, 1983; Collier *et al.*, 1984; Nuttall and Gannon, 1991; Sheard *et al.*, 2004). This effect can be seen in the current study as the flaxseed muffins contained higher fat, protein and fibre content.

Moreover the flaxseed muffins contained a lower amount of starch compared to the control muffin which may explain the results of the lower GI value in the flaxseed muffins compared to the control muffin (see Section 3.2.4, Table 3.1).

TPC can affect the GI results as it can inhibit the starch breakdown to glucose by affecting the digestive enzymes such as α -amylase and α -glucosidase (McDougall and Stewart, 2005). This may result in reducing the sugar released from the starch in the food and this would reduce the RDS and leads to lower GI. The lower GI value of the ground flaxseed muffins compared to the whole and the control muffins (see Chapter 2 section 2.3.2) may be explained by the higher amount of TPC with 518.8 μg GAE/g compared to 414.4 μg GAE/g for the whole flaxseed muffins and 260 μg GAE/g for the control muffins.

Food processing such as milling, grinding and rolling can also affect the GI results, as they have different impacts on blood glucose level and on GI values. Such processing breaks the food down into smaller pieces that are easier for enzymes to digest. During milling and grinding, the outer coat of the grain is removed, which reduces the time needed to digest the starch inside the food (Philippou, 2017).

This hypothesis is not consistent with the GI results for flaxseed muffins, where the ground flaxseed muffins resulted in a lower GI compared to the whole flaxseed muffins. This can be explained by reference to the TPC in the ground flaxseed muffins, which was higher than that of the whole flaxseed muffins (see Section 2.3.1) as the TPC had an impact on the starch digestion. Another explanation could be the necessity of chewing the whole seeds, which would shred the seed coat and expose nutrients inside the seed to digestive enzymes, making for easier digestion (Morris,

2007). Referring back to the study in Chapter 2, this effect helps to explain the lower (but not significantly different) RDS observed for the ground flaxseed muffins compared to the whole flaxseed muffins. However, the effect on starch digestibility was not large, as using whole flaxseed still lowered the amount of RSD.

All the results from previous studies and the current study show that blood glucose levels were lower in the test food, whether this was bread, muffins or a glucose solution fortified with fibre, than in standard glucose. All the test foods contained dietary fibre and resistant starch, which have different digestive pathways compared with other types of CHO. Therefore, fibre would be fermented by the colonic bacteria, producing short chain fatty acids, and this can be a key factor in the modulation of glucose and fat metabolism in the liver (Thorburn *et al.*, 1993).

3.5 Summary

This study has provided important insights into the effect of adding different forms of flaxseed to bakery products, specifically muffins, on GI. The results showed that flaxseed was effective in lowering the GI and that it is possible to enhance GI in bakery products by adding functional ingredients (such as flaxseed). This may provide a better option for incorporating these foods into the diets of both healthy people and those with T2D. This study focused on the effects on GI; however there is also a need for research on the effects of flaxseed on glucose and insulin levels to provide a better understanding of the effect on the blood glucose profile. The subsequent study was therefore aim to support these results and provide data on insulin and satiety.

Chapter 4 : The effect of whole and ground flaxseed on glycaemic and insulinaemic response

4.1 Justification and aims

Flaxseed contains bioactive compounds such as lignans, dietary fibre and ALA (Goyal *et al.*, 2013), compounds that may have an impact on the GR/IR. Studies of flaxseed protein and lignans have suggested that they play a role in lowering blood glucose as they can induce insulin excretion, which results in a lowered GR (Nuttall *et al.*, 1984; Pan *et al.*, 2007).

This role in lowering GR is supported by a study that investigated the effect of flaxseed on GR. Using flaxseed as a powder (10 g per day) in patients with diabetes for one month, Mani *et al.* (2011) found a lowered fasting blood glucose (by 19.7 %) and also a lowered HbA1c (by 15.6 %).Further support for this hypothesis comes from other studies of role of flaxseed fibre, which have shown it can slow sugar release and lower blood glucose levels leading to longer time periods of normal blood glucose maintenance (Thakur *et al.*, 2009). Studies carried out by Cunanan *et al.*, (1993) and Cunanan *et al.* (1995) also support this as they showed flaxseed supplementation could lower postprandial blood glucose levels compared to wheat. Overall these studies provide a solid foundation on which to start building the current study.

Bakery products are considered to be suitable for fortification, especially with functional foods, as they are consumed regularly and they are easy to fortify for regular consumption as well as for clinical trials (Lima, Guraya and Champagne (2002; Kadam and Prabhasankar, 2010).

Muffins have been shown to be a widely available and a popular breakfast or afternoon snack, meaning any functional food additions would reach a large audience (Matos, Sanz, and Rosell, 2014). Muffins are a suitable bakery product for the addition of functional components as they are generally ready-to-eat, high calorie, sweet products that have a good taste, a soft texture and are affordable; they are enjoyed by all age groups and come in different varieties to suit different tastes with a soft texture (Goswami *et al.*, 2015). Studies have shown that muffins can be an appropriate vehicle for fortification; for example they can allow participants to consume up to 50 g/day of flaxseed (Cunnane *et al.*, 1993).

Most flaxseed-based studies have focused on patients with hyperlipidaemia or diabetes, but the results of the studies suggest there may be benefits to encouraging consumption of flaxseed in healthy individuals. For example, flaxseed could be used as a supplement to help weight management due to its high fibre content (Kristensen *et al.*, 2012; Gutte *et al.*, 2015). Overall further studies are needed to fully investigate the effect on key metabolic risk factors of using flaxseed as a supplement in different products (e.g. bakery products).

Within the literature, the flaxseed amount, as regards its effect on GR, varied from small amounts, such as 10 g and 13 g of flaxseed in the studies of Mani *et al.* (2011) and Hutchins *et al.* (2013) respectively, to the higher amounts of 30 g and 50 g per day in the studies of Wu *et al.* (2010) and Cunnane *et al.* (1995) respectively. Flaxseed in all of these studies was effective in lowering blood glucose level among different with different-participant groups (healthy, pre diabetic, metabolic syndrome and T2 diabetic).

The amount of flaxseed added was chosen with reference to Wu *et al.* (2010), who showed that 30 g of milled flaxseed was effective in lowering blood glucose; this amount was also effective with regard to satiety, one of the outcomes used in the study by Monteiro *et al.* (2016), which found that adding 30g of flaxseed was effective in increasing the satiety.

Another factor on which the choice of the 30 g was based was the effect on sensory evaluation, as there was a need for the muffin to be acceptable. In this study 30 g of flaxseed equals a 48% replacement of flour. Lipilina and Ganji (2009) showed that replacing 50% of the flour with ground flaxseed in a muffin resulted in the most acceptable sensory evaluation compared to the control bread with wheat flour. Ground flaxseed was the best option to add to bakery products as it enhanced the sensory attributes and could be added in high amounts (up to 50%) without any negative effects (Alpers and Sawyer-Mores 1996).

Within the literature, to our knowledge, currently there are no studies that compare the effects of using whole and ground flaxseed in bakery products (for example in muffins) on GR and IR. The primary aim of this study was to determine the effect of flaxseed in different forms (whole and ground) on GR, IR and satiety. This secondary aim of the study was to assess the effect of adding flaxseed to muffins on the sensory attributes of appearance, colour, flavour, texture and overall acceptability.

4.2 Methods

4.2.1 Participants

Healthy male and female adults aged between 18-60 years were recruited to this study at Oxford Brookes University through advertisements and personal communications between February 2016 and September 2016. The inclusion criteria were similar to those for the study of determination of GI in Chapter 3 (see Section 3.2.1).

4.2.2 Study design

The study was a randomised, cross over repeated-measures design. Randomisation of the order of food tested by participants was carried out using a random number generator website (<https://www.randomizer.org>). This study involved four visits: the first was the screening visit and the remaining three visits were for testing the different muffins (control muffin, whole flaxseed muffin and ground flaxseed muffin) and their effect on GR and IR. Ethical approval for the study was obtained from the University Research Ethics Committee (UREC Registration No: 150959) (Appendix 3.a).

A PIS (Appendix 3.b) was sent to the interested participants by email prior recruitment. All details of the screening visit were similar to those for the study of determination of GI in Chapter 3 (see Section 3.2.2). If they agreed to take part in the study, they were then asked to sign a consent form (Appendix 3.c)

Each test session lasted for approximately 2 hours and was conducted in the morning (starting between 07:00 and 09:30 am) at Oxford Brookes Centre for Nutrition and Health, Oxford Brookes University. During each test session, participants were required to consume a control or flaxseed muffin and capillary blood samples were taken for the determination of glucose and insulin.

4.2.3 Test foods

The muffins (flaxseed and control) were prepared using a standard recipe (see Section 2.2.1) with 15 g of flaxseed added per muffin. Each participant was required to consume two muffins, containing a total of 30 g of flaxseed, as this amount of flaxseed has been found to be safe, acceptable and effective in lowering GR based on studies by Taylor *et al.* (2010); Hutchins *et al.* (2013); Rodriguez-Leyva *et al.* (2013)

4.2.4 Glycaemic response

The method used to determine the GR to the muffins was similar to that described in Chapter 3 (see Section 3.2.5).

4.2.5 Insulinaemic response

For IR determination, 300 μ l of finger prick capillary blood was taken at each test time point (-5, 0, 15, 30, 45, 60, 90 and 120 minutes). Unistik 3 single-use lancing devices (Owen Mumford, UK) were used to make finger pricks and EDTA-coated tubes were used to collect the blood (Becton, Dickinson U.K. Limited, UK). Samples were centrifuged for 10 minutes at 4000 rpm (Sigma 1-14 microfuge, Sigma Laborzentrifugen, Germany) and 200 μ l of the supernatant plasma was placed into

1.5ml Eppendorffs, then frozen at -40°C until analysis. The plasma sample insulin concentrations (μ U/ml) were determined using an electrochemiluminescence immunoassay and an automated analyzer (Cobas® E411; Roche diagnostics, UK). This system has been shown to be reliable for the determination of plasma insulin concentrations (Siahanidou, *et al.*, 2011). The iAUC was used trapezoid method to calculate the IR without the area beneath the baseline (FAO/WHO, 1998) but with the area above the fasting level.

4.2.6 Glycaemic and insulinaemic response according to gender

Previous analysis of blood glucose and insulin data according to gender implies that insulin sensitivity differs with gender (Masharani,Goldfine and Youngren, 2009). When comparing men with women of the same age, it is found that women have lower skeletal muscle mass, more circulating free fatty acids (FFA), a greater adipose tissue mass as well as greater intramyocellular lipid content. All of these factors can boost insulin resistance in women compared to men (Frias *et al.*, 2001). However, glucose disposal in women shows comparable results to that of men and that can be explained with reference to the improved glucose efficiency in women (Basu *et al.*, 2006; Masharani,Goldfine and Youngren, 2009) Improved glucose efficiency can be defined as the capability of glucose to stimulate its own disposal in an insulin-dependent way (Basu *et al.*, 2006).

Furthermore, women have a tendency towards improved postprandial insulin and C-peptide concentrations, irrespective of similar plasma glucose, following a meal test. There is a degree of uncertainty regarding the means of this facilitated glucose homeostasis in women, as compared with men (Basu *et al.*, 2006).

4.2.7 Satiety

The participant's subjective feelings of satiety/hunger were recorded on a 7-point anchored scale, which was used to describe the satiety/hunger at the time points 0, 15, 30, 45, 60, 90 and 120 minutes for all of the test days (Flint, *et al.*, 2000); ratings run between 6 = extremely full and 0 = extremely hungry (Appendix 3.d). The score was used to calculate the total AUC for 60, 90 and 120 min using the trapezoid method.

4.2.8 Sensory evaluation

Participants evaluated the sensory acceptability for each muffin type (Appendix 3.e). This was recorded on a 9-point anchored scale for appearance, colour, flavour, texture and overall acceptability, where 9 = like extremely and 1 = dislike extremely (Poste *et al.*, 1991)

4.2.9 Gastrointestinal symptoms questionnaire

A gastrointestinal symptoms questionnaire (Appendix 3.f) was completed by each participant for 24 hours after each test day. The questionnaire was used to determine if the participants experienced any gastrointestinal symptoms and consisted of questions about the severity of the GI symptoms rated 0-4, where 0 = “no complaints” and 4 = the highest severity meaning “symptoms unbearable”. This questionnaire was adapted from the questionnaire used by Bovenschen *et al.* (2006).

4.2.9 Statistical analysis

The required sample size was estimated to be 32 to be able to detect a 20 % reduction in blood glucose iAUC considering that the confidence level was 95 % for a medium effect size, with $\alpha=0.05$ (two-tailed) using G*power (Version 3.0.10). The power of the study was 90 % and the number of participants was based on the previous study by Lu *et al.* (2017).

The required sample size for the analysis of gender difference was based on the difference in the mean blood glucose AUC of 10 mmol/l and a mean standard deviation of 5, considering that the confidence level was 95 % for a medium effect size, with $\alpha=0.05$ (two-tailed) using G*power (Version 3.0.10). The power of the study was 90 % and the minimum required sample size was 11 participants (Clegg *et al.*, 2011).

Data were analysed using the SPSS (Statistical Package for the Social Sciences) v25.0 (Chicago, USA). All data have been presented as mean and SD and SEM. The Shapiro-Wilk test was performed to investigate the normality of the data; where data were not normally distributed, non-parametric tests were used. The independent t-test (for parametric data) and Mann-Whitney test (for non-parametric data) were used to determine the differences in the participant's baseline characteristics according to the gender.

The repeated measures ANOVA using a Bonferroni for multiple comparisons (for parametric data) and Friedman and Wilcoxon tests (for non-parametric data) were used to determine differences in the blood glucose and insulin iAUC, the change from

the baseline for blood glucose and insulin concentrations over 2 hours, peak blood glucose/insulin values, time to peak, sensory evaluation and gastrointestinal symptoms between the three muffin types. A Bonferroni correction was used to correct for multiple comparisons of the results in the change from the baseline for blood glucose and insulin. For satiety AUC, a one-way ANOVA using the baseline as a covariate in the analysis was performed to determine the differences between the muffin types.

The results for iAUC, peak values, and time to peak for the blood glucose and insulin divided by gender were analysed using a two-way repeated measures ANOVA of variance (muffin types x gender) to study the differences between males and females. Statistical significance was set at ($p < 0.05$) for all analysis except the results of the change from the baseline for blood glucose and insulin ($p < 0.008$) for those results after the Bonferroni correction.

4.3 Results

4.3.1 Participants characteristics

Thirty-two participants (eleven males, twenty one females) were recruited to the study. The baseline characteristics of the participants are shown in (Table 4.1). There was a significant difference between male and female for the weight and body fat results ($p = 0.002$ and < 0.001 respectively).

Table 4.1. Baseline characteristics of thirty two participants (mean $\pm$ SD)

	All (n 32)	Male (n11)	Female (n21)	<i>p</i> value
Age (y)	29.5 $\pm$ 8.6	30.4 $\pm$ 5.1	28.9 $\pm$ 6.3	0.289
Height (m)	1.7 $\pm$ 0.1	1.7 $\pm$ 0.1	1.7 $\pm$ 0.1	0.056
Weight (kg)	68.1 $\pm$ 10.2	73.0 $\pm$ 8.0	63.3 $\pm$ 11.1	0.002
BMI (kg/m ²)	23.8 $\pm$ 2.3	24.6 $\pm$ 2.8	23.1 $\pm$ 3.1	0.165
Body fat %	21.3 $\pm$ 5.9	13.5 $\pm$ 5	29.2 $\pm$ 6.9	< 0.001
Fasting blood glucose (mmol/l)	4.9 $\pm$ 0.1	4.7 $\pm$ 0.6	4.8 $\pm$ 0.5	0.695

p value based on the independent t-test or Mann Whitney test .

4.3.2 Glycaemic response

4.3.2.1 Absolute blood glucose

The results for the absolute blood glucose levels over time (Table 4.2) showed that for the flaxseed muffins (whole and ground flaxseed muffins) the values were lower than the control, especially at 30, 45, 60 min post consumption.

Table 4.2. Blood glucose measurements (mmol/l) for each muffin type(mean $\pm$ SD)

Time (min)	CM	WFM	GFM
0	4.7 $\pm$ 0.5	4.7 $\pm$ 0.5	4.8 $\pm$ 0.5
15	5.4 $\pm$ 0.7	5.2 $\pm$ 0.7	5.3 $\pm$ 0.6
30	6.7 $\pm$ 0.8	6.3 $\pm$ 0.7	6.3 $\pm$ 1.0
45	6.3 $\pm$ 1.1	5.9 $\pm$ 1.0	5.9 $\pm$ 1.0
60	5.8 $\pm$ 1.0	5.3 $\pm$ 1.0	5.2 $\pm$ 1.0
90	5.4 $\pm$ 0.7	4.8 $\pm$ 0.7	5.2 $\pm$ 0.7
120	5.1 $\pm$ 0.6	4.8 $\pm$ 0.5	5.0 $\pm$ 0.6

CM = control muffin; WFM = whole flaxseed muffin; GFM = ground flaxseed muffin

4.3.2.2 Blood glucose iAUC

The results for blood glucose iAUC and the post-hoc multiple pair-wise comparisons between the muffin types (Tables 4.3- 4.4) showed that the iAUC at 90 and 120 min

were significantly lower in the flaxseed muffins compared to the control muffin ($p < 0.001$). There was no significant difference in the blood glucose iAUC between the two flaxseed muffins.

Table 4.3. Blood glucose iAUC (mmol.min/l) for each muffin type (mean $\pm$ SD)

	CM	WFM	GFM	<i>p</i> value
iAUC 60	76.6 $\pm$ 30.7	60.0 $\pm$ 30.4 ^a	57.5 $\pm$ 25.0 ^a	< 0.001
iAUC 90	106.5 $\pm$ 43.6	77.5 $\pm$ 44.9 ^a	76.3 $\pm$ 42.2 ^a	< 0.001
iAUC 120	126.4 $\pm$ 53.0	87.4 $\pm$ 50.0 ^a	90.0 $\pm$ 54.5 ^a	< 0.001

^a Significant difference ($p < 0.05$) compared to control muffins.

CM = control muffin; WFM = whole flaxseed muffin; GFM = ground flaxseed muffin.

p values based on the repeated measures ANOVA using Bonferroni for multiple comparisons or Friedman and Wilcoxon tests.

Table 4.4. Post hoc analysis of blood glucose iAUC at 60, 90 and 120 mins for each muffin type

iAUC 60 (mmol.min/l)			
	CM	WFM	GFM
CM		0.002	0.001
WFM	0.002		1.000
GFM	0.001	1.000	
iAUC 90 (mmol.min/l)			
	CM	WFM	GFM
CM		< 0.001	< 0.001
WFM	< 0.001		0.779
GFM	< 0.001	0.779	
iAUC 120 (mmol.min/l)			
	CM	WFM	GFM
CM		< 0.001	< 0.001
WFM	< 0.001		0.940
GFM	< 0.001	0.940	

CM = control muffin; WFM = whole flaxseed muffin; GFM = ground flaxseed muffin.

p values based on the repeated measures ANOVA using Bonferroni for multiple comparisons or Friedman and Wilcoxon tests.

The results for the change in blood glucose concentration from baseline (Figures 4.1) showed that there were differences between the flaxseed muffins types and the control muffin. The differences were significant ($p < 0.008$) for time points 60 between both flaxseed muffins and the control, while at 90 minutes the difference was between the whole and the control muffins. These results are consistent with the results of the iAUC values (Table 4.3). The addition of flaxseeds had the effect of lowering blood glucose response for both flaxseed muffin types compared to the control muffin.

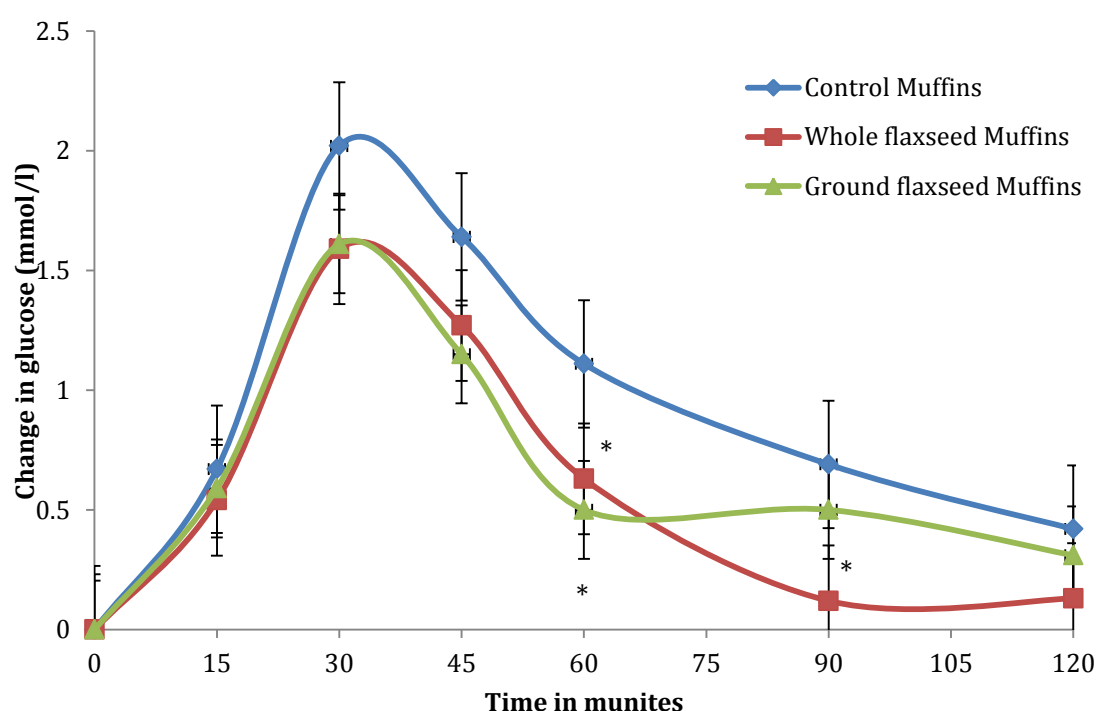


Figure 4.1. Change in blood glucose concentration (mmol/l) from baseline (0 min) over time according to muffin type.

Values mean $\pm$ SEM (n=32).

*Significant differences $p < 0.008$ (after Bonferroni correction) compared to control muffin.

4.3.2.3 Glucose peak values and time to peak

The results for the peak glucose values (Table 4.5) indicated that the values for the flaxseed muffins were lower than the control muffin; the difference was statically

significant ($p > 0.05$) between the control and both flaxseed muffins. Regarding the time to peak, the difference was not significant between all the muffin types.

Table 4.5. Blood glucose peak values and time to peak for each muffin type (mean $\pm$ SD)

	CM	WFM	GFM	<i>p</i> value
Peak (mmol/l)	6.9 $\pm$ 0.8	6.4 $\pm$ 0.8 ^a	6.5 $\pm$ 0.8 ^a	< 0.001
Time to peak (min)	39.0 $\pm$ 11.6	37.0 $\pm$ 17.3	39.0 $\pm$ 20.2	0.566

^a Significant difference ($p < 0.05$) compared to control muffins

CM = control muffin; WFM = whole flaxseed muffin; GFM = ground flaxseed muffin.

p values based on the repeated measures ANOVA using Bonferroni for multiple comparisons or Friedman and Wilcoxon tests.

The statistical differences between the peak values were analysed using post-hoc multiple pair-wise comparisons (Table 4.6). The results show that the *p* values were < 0.001 and 0.001 for the whole and the ground flaxseed muffins respectively compared to the control muffin.

Table 4.6: Post hoc analysis of peak blood glucose values for each muffin type

	CM	WFM	GFM
CM		< 0.001	0.001
WFM	< 0.001		1.000
GFM	0.001	1.000	

CM = control muffin; WFM = whole flaxseed muffin; GFM = ground flaxseed muffin.

p values based on the repeated measures ANOVA using Bonferroni for multiple comparisons or Friedman and Wilcoxon tests.

4.3.3 Insulinaemic response

4.3.3.1 Absolute insulin values

The results for the absolute insulin levels over time (Table 4.7) showed that for all flaxseed muffins (whole and ground) the insulin levels was lower than the control muffin, specifically at 30, 45, 60 and 120 min post consumption. This is consistent with the GR findings (see Table 4.2).

Table 4.7. Insulin values (pmol/l) for each muffin type (mean $\pm$ SD)

Time (min)	CM	WFM	GFM
0	10.2 $\pm$ 4.6	9.5 $\pm$ 4.3	11.1 $\pm$ 7.0
15	47.8 $\pm$ 34.5	33.7 $\pm$ 18.4	35.3 $\pm$ 17.7
30	81.8 $\pm$ 45.8	60.1 $\pm$ 32.1	55.6 $\pm$ 27.9
45	69.3 $\pm$ 41.1	51.9 $\pm$ 28.4	45.2 $\pm$ 25.8
60	60.6 $\pm$ 35.8	36.8 $\pm$ 20.4	35.8 $\pm$ 19.3
90	41.4 $\pm$ 20.9	26.6 $\pm$ 15.4	31.0 $\pm$ 25.2
120	30.0 $\pm$ 17.1	18.9 $\pm$ 13.0	19.3 $\pm$ 12.9

CM = control muffin; WFM = whole flaxseed muffin; GFM = ground flaxseed muffin.

4.3.3.2 Blood insulin iAUC

The results for iAUC for IR and the post-hoc multiple pair-wise comparisons between the muffin types (Table 4.8-4.9) showed that the iAUC at 60, 90 and 120 min were significantly lower in both types of flaxseed muffins compared to the control muffin ($p < 0.001$). There was no significant difference in the insulin iAUC between the two types of flaxseed muffins.

Table 4.8. Insulin iAUC (pmol.min/l), for each muffin type (mean $\pm$ SD)

	CM	WFM	GFM	<i>p</i> value
iAUC 60	2921.2 $\pm$ 1514.3	2094.2 $\pm$ 1184.9 ^a	1890.2 $\pm$ 1365.8 ^a	< 0.001
iAUC 90	4023.2 $\pm$ 2150.7	2629.0 $\pm$ 1363.3 ^a	2394.4 $\pm$ 1417.3 ^a	< 0.001
iAUC 120	4764.0 $\pm$ 2545.0	3062.9 $\pm$ 1625.0 ^a	2873.2 $\pm$ 1809.7 ^a	< 0.001

^a Significant difference ($p < 0.05$) compared to control muffin

CM = control muffin; WFM = whole flaxseed muffin; GFM = ground flaxseed muffin.

p values based on the repeated measures ANOVA using Bonferroni for multiple comparisons or Friedman and Wilcoxon tests.

Table 4.9. Post hoc analysis of insulin iAUC at 60, 90 and 120 mins for each muffin type

iAUC 60 (pmol.min/l)			
	CM	WFM	GFM
CM		< 0.001	< 0.001
WFM	< 0.001		0.085
GFM	< 0.001	0.085	
iAUC 90 (pmol.min/l)			
	CM	WFM	GFM
CM		< 0.001	< 0.001
WFM	< 0.001		0.184
GFM	< 0.001	0.184	
iAUC 120 (pmol.min/l)			
	CM	WFM	GFM
CM		< 0.001	< 0.001
WFM	< 0.001		0.443
GFM	< 0.001	0.443	

CM = control muffin; WFM = whole flaxseed muffin; GFM = ground flaxseed muffin.

p values based on the repeated measures ANOVA using Bonferroni for multiple comparisons or Friedman and Wilcoxon tests.

The results of the change in insulin concentration (Figures 4.2) from baseline showed that there were differences between the flaxseed muffins types compared to the control muffin. The differences were significant ($p < 0.008$) for the time points 30, 45, 60, 90 and 120 min after consumption. These results are consistent with the results of

the insulin iAUC values (Table 4.8). The addition of flaxseed had the effect of lowering IR for both flaxseed muffin types compared to the control muffin.

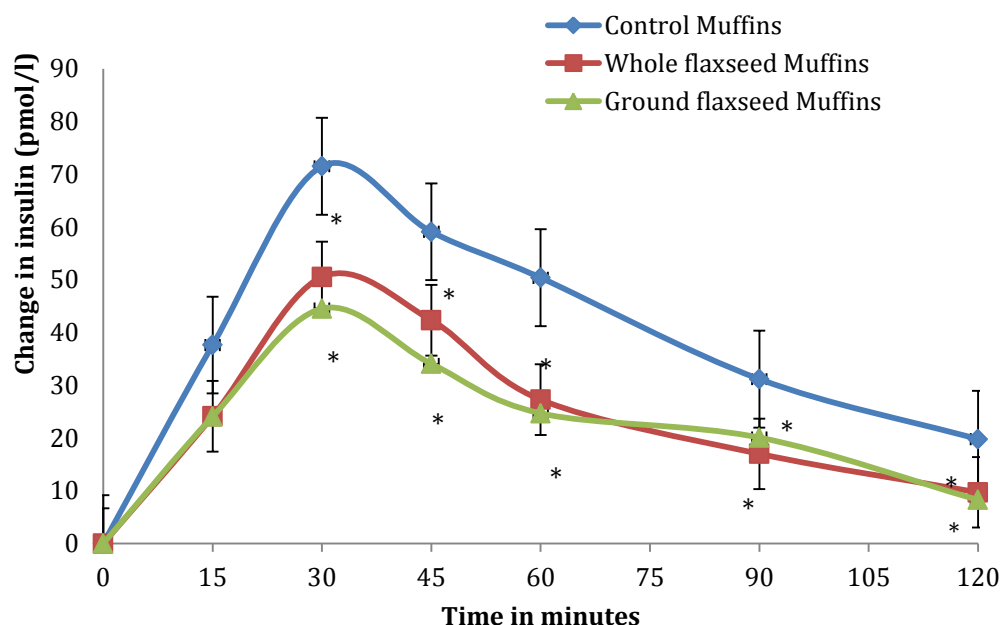


Figure 4.2. Change in insulin concentration from the baseline (0 min) (pmol/l) over time for each muffin type.

Values mean $\pm$ SEM (n=32)

*Significant differences $p < 0.008$ (after Bonferroni correction) compared to control muffin.

4.3.3.3 Insulin peak and time to peak

The results for the peak insulin values (Table 4.10) indicated that the values for the different flaxseed types were lower than control and the difference was significant between both flaxseed type and the control muffin ($p < 0.001$). Regarding to time to peak, the difference was not significant between all muffin types.

Table 4.10. Insulin peak values and time to peak for each muffin type (mean $\pm$ SD)

	CM	WFM	GFM	<i>p</i> value
Peak (pmol/l)	92.9 $\pm$ 41.4	68.9 $\pm$ 31.2 ^a	62.9 $\pm$ 25.2 ^a	< 0.001
Time to peak (min)	37.5 $\pm$ 13.1	34.22 $\pm$ 11.5	36.5 $\pm$ 25.5	0.438

^a Significant difference ($p < 0.05$) compared to control muffins

CM = control muffin; WFM = whole flaxseed muffin; GFM = ground flaxseed muffin.

p values based on the repeated measures ANOVA using Bonferroni for multiple comparisons or Friedman and Wilcoxon tests.

The results for the post-hoc multiple pair-wise comparisons between the muffin types for the insulin peak values (Table 4.11) showed that the *p* values were < 0.001 for whole and ground flaxseed muffins compared to the control muffins.

Table 4.11. Post hoc analysis of peak blood insulin values for each muffin type

	CM	WFM	GFM
CM		< 0.001	< 0.001
WFM	< 0.001		0.112
GFM	< 0.001	0.112	

CM = control muffin; WFM = whole flaxseed muffin; GFM = ground flaxseed muffin.

p values based on the repeated measures ANOVA using Bonferroni for multiple comparisons or Friedman and Wilcoxon tests.

4.3.4 Glycaemic response according to gender

The results of the iAUC for blood glucose for each gender for each muffin type (Table 4.12) indicated that there were no significant interactions between gender and muffins type at 60 minutes ($F(2, 90) = 0.811, p = 0.448$), 90 minutes ($F(2, 90) = 0.877, p = 0.420$), or 120 minutes ($F(2, 90) = 1.406, p = 0.250$).

Table 4.12. Gender difference in blood glucose iAUC (mmol.min/l), for each muffin type (mean $\pm$ SD) M=11, F=21

		<i>p</i> value	CM	WFM	GFM
iAUC 60	M	0.448	87.5 $\pm$ 24.7	64.5 $\pm$ 24.2	55.7 $\pm$ 27.6
	F		71.0 $\pm$ 32.5	58.1 $\pm$ 33.6	58.5 $\pm$ 24.2
iAUC 90	M	0.420	121.2 $\pm$ 42.0	83.3 $\pm$ 38.1	71.0 $\pm$ 43.4
	F		99.0 $\pm$ 43.2	74.5 $\pm$ 48.7	79.1 $\pm$ 42.5
iAUC 120	M	0.250	146.4 $\pm$ 51.5	93.2 $\pm$ 43.2	79.5 $\pm$ 49.0
	F		116.0 $\pm$ 52.0	84.5 $\pm$ 55.4	95.6 $\pm$ 57.6

CM = control muffin; WFM = whole flaxseed muffin; GFM = ground flaxseed muffin
M = male, F = female.

p values based two-way repeated measures ANOVA of variance to assess the interaction for (muffin types x gender).

The results for the glucose peak and time of the peak for each muffin according to gender indicated that there was no difference between males and females for each muffin type. The results for the peak glucose values (Table 4.13) showed that there were no significant interactions between gender and muffins type for the blood glucose peak ($F(2, 90) = 0.733$, $p = 0.465$), as well as time to peak ($F(2, 90) = 1.598$, $p = 0.208$).

Table 4.13. Gender difference in blood glucose peak values and time to peak for each muffin type (mean $\pm$ SD) M=11, F=21.

		<i>p</i> value	CM	WFM	GFM
Peak (mmol/l)	M	0.465	7.3.5 $\pm$ 0.7	6.5 $\pm$.07	6.5 $\pm$.0.9
	F		6.8 $\pm$ 0.84	6.5 $\pm$ 0.9	6.5 $\pm$ 0.8
Time to peak(min)	M	0.208	39.5 $\pm$ 12.1	35.5 $\pm$ 7.6	30 $\pm$.9.5
	F		38.2 $\pm$ 11.6	37.5 $\pm$ 21	43.6 $\pm$ 22.8

CM = control muffin; WFM = whole flaxseed muffin; GFM = ground flaxseed muffin

M = male, F =female *p* values* based two-way repeated measures ANOVA of variance to assess the interaction for (muffin types x gender).

4.3.5 Insulinaemic response according to gender

The results of the iAUC for blood insulin for gender for each muffin type (Table 4.12) indicated that there were no significant interactions between gender and muffins type at 60 minutes ($F(2, 90) = 0.027$, $p = 0.973$), 90min ($F(2, 90) = 0.009$, $p = 0.991$), or 120min ($F(2, 90) = 0.034$, $p = 0.967$).

Table 4.14. Gender difference in insulin iAUC (pmol/.min/l), for each muffin type (mean $\pm$ SD) M=11, F=21.

		<i>p</i> value	CM	WFM	GFM
iAUC 60	M		2539.4 $\pm$ 1067.8	1725.8 $\pm$ 899.8	1421.2 $\pm$ 933
	F	0.973	3121.2 $\pm$ 1691.9	2287.3 $\pm$ 1287.6	2136 $\pm$ 1507.3
iAUC 90	M		3629 $\pm$ 1588.8	2249.9 $\pm$ 1171.1	1942.1 $\pm$ 1331
	F	0.991	4229.7 $\pm$ 2403.6	2827.6 $\pm$ 1440.4	2631.3 $\pm$ 1434.1
iAUC 120	M		4376.6 $\pm$ 1948.8	2532.7 $\pm$ 1271.2	2316.5 $\pm$ 1548.6
	F	0.967	3340.6 $\pm$ 1746.3	3340.7 $\pm$ 1746.3	3165 $\pm$ 1902.1

CM = control muffin; WFM = whole flaxseed muffin; GFM = ground flaxseed muffin
M = male, F = female.

p values based two-way repeated measures ANOVA of variance to assess the interaction for (muffin types x gender).

The results for the insulin peak and time of the peak for each muffin according to gender indicated that there was no difference between males and females for each muffin type. The results for the peak glucose values (Table 4.13) showed that there were no significant interactions between gender and muffin type for the blood insulin peak ($F(2, 90) = 0.604$, $p = 0.549$), as well as time to peak ($F(2, 90) = 0.207$, $p = 0.813$).

Table 4.15. Insulin peak values and time to peak for each muffin type (mean $\pm$ SD) M=11, F=21.

		<i>p</i> value	CM	WFM	GFM
Peak	M	0.549	94 $\pm$ 44.8	65.5 $\pm$ 30.4	56.6 $\pm$ 24.7
	F		92.4 $\pm$ 40.7	70.7 $\pm$ 32.3	66.4 $\pm$ 25.4
Time peak	M	0.813	36.8 $\pm$ 14	36.8 $\pm$ 10.3	39.6 $\pm$ 26.2
	F		37.9 $\pm$ 13.1	32.9 $\pm$ 12.2	35 $\pm$ 25.7

CM = control muffin; WFM = whole flaxseed muffin; GFM = ground flaxseed muffin

M = male, F =female.

p values based two-way repeated measures ANOVA of variance to assess the interaction for (muffin types x gender).

4.3.6 Satiety

The results for the AUC for satiety (Table 4.13) and satiety rating for each time point (Figure 4.3) showed that the AUC at 60, 90 and 120 min post consumption was not significantly different ($p > 0.05$) between the types of muffins (control, whole and ground flaxseed muffins).

Table 4.16. Satiety AUC (mm*h) for each muffin type (mean $\pm$ SD)

	CM	WFM	GFM	<i>p</i> value
AUC 60	261.8 $\pm$ 46.0	277.2 $\pm$ 34.8	269.5 $\pm$ 45.4	0.398
AUC 90	378.9 $\pm$ 61.8	407.6 $\pm$ 61.5	394.0 $\pm$ 80.1	0.277
AUC 120	482.1 $\pm$ 82.4	524.2 $\pm$ 90.5	499.5 $\pm$ 118.5	0.252

CM = control muffin; WFM = whole flaxseed muffin; GFM = ground flaxseed muffin.

p values based on the repeated measures ANOVA using Bonferroni for multiple comparisons OR Friedman and Wilcoxon tests

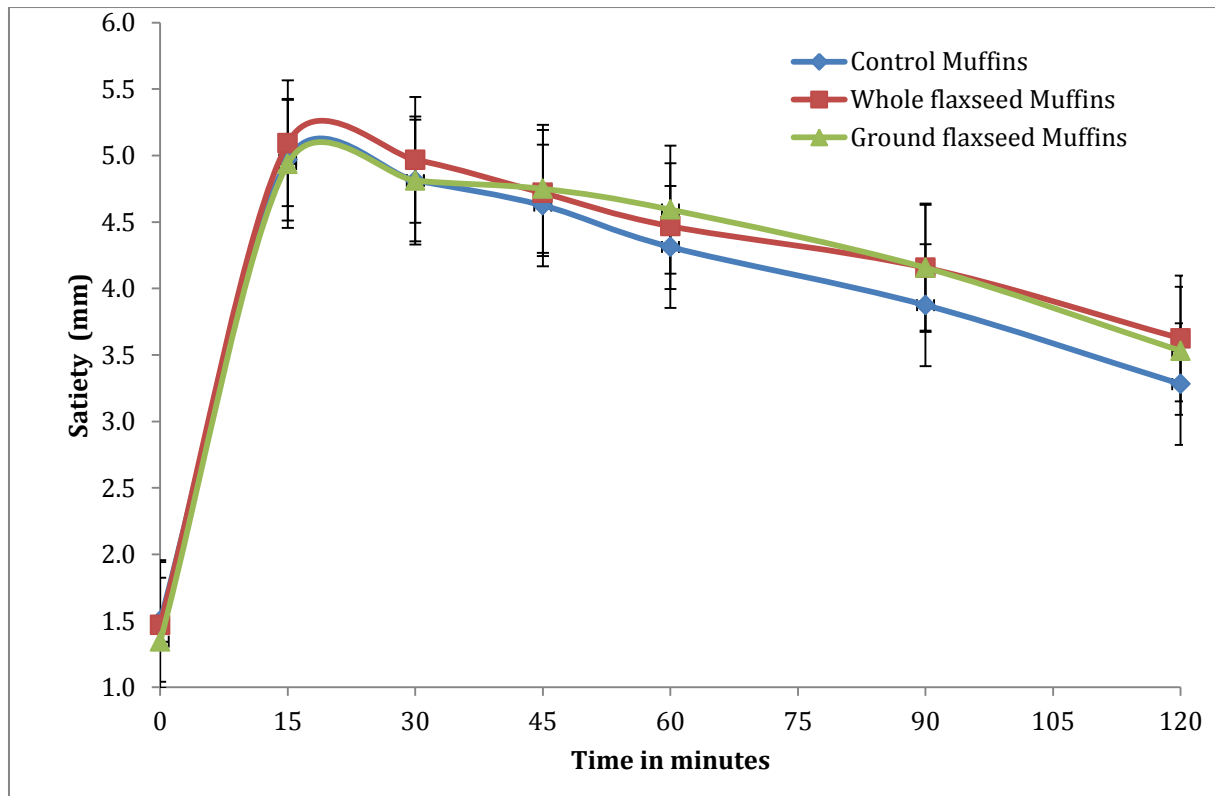
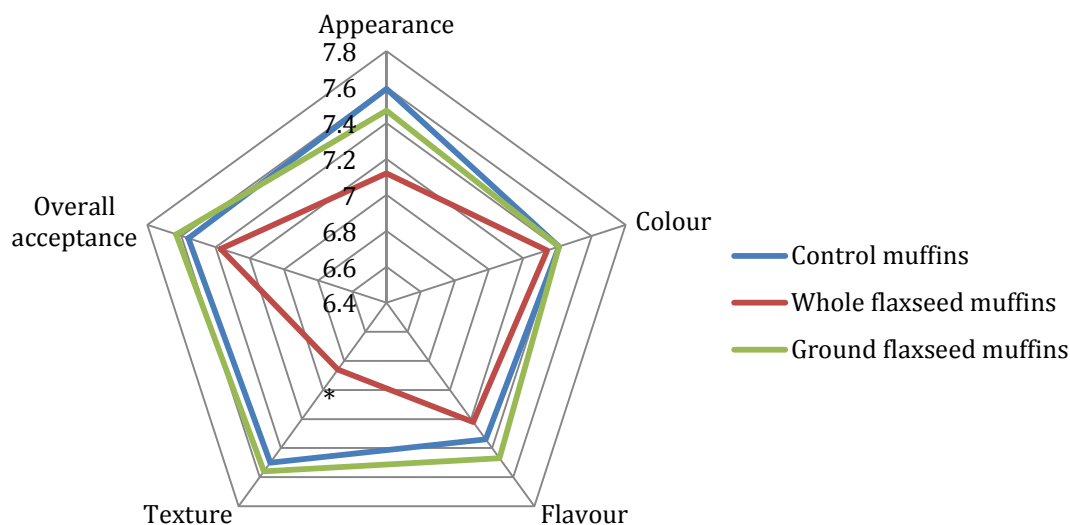


Figure 4.3. Satiety rating for each time point for each muffin type.

4.3.7 Sensory evaluation

The sensory evaluation of the muffins (appearance, colour, flavour, texture and overall acceptability) (Figure 4.3) showed that there was no significant difference in the attributes for all muffin types except for texture, which was significantly different ($p = 0.049$) between the whole and ground flaxseed muffins. Overall, the results showed a high rate of acceptance by the participants as the scores for that attribute were mostly 'like moderate'.

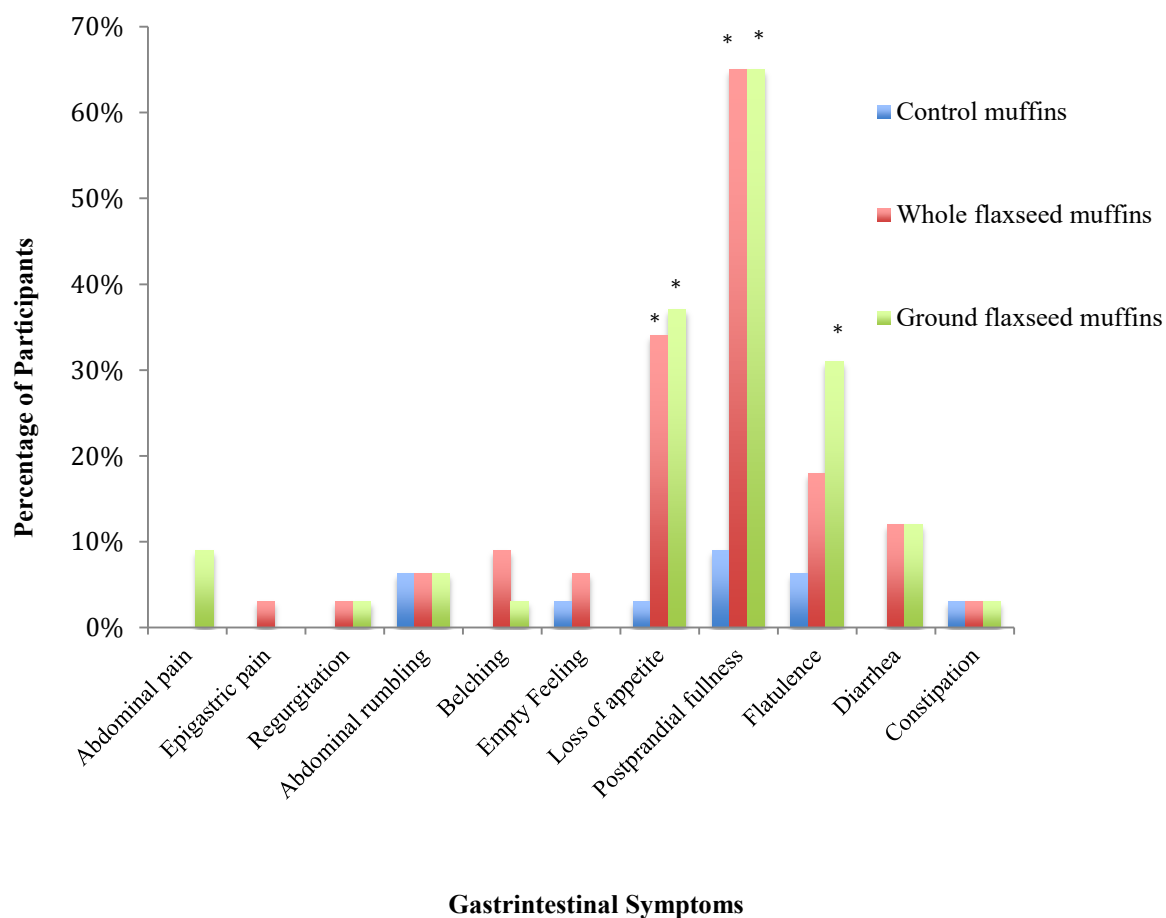


.Figure 4.4. Sensory evaluation for each muffin type.

* Significant difference in texture ($p < 0.05$) compared to ground flaxseed muffins.

4.3.8 Gastrointestinal symptoms

The results for the gastrointestinal symptoms at 24 h post consumption (Figure 4.4) showed that loss of appetite, postprandial fullness and flatulence were the most frequent gastrointestinal symptoms reported when participants consumed the whole and ground flaxseed muffins. The difference was significantly higher ($p > 0.05$) for both flaxseed muffins compared to the control muffin.



.Figure 4.5. Gastrointestinal symptoms 24 hour post consumption for each muffin type
 *Significant difference ($p < 0.05$) compared to control muffins

4.4 Discussion

The results of this study showed that the muffins that contained flaxseed had a lowering effect on GR and IR over 2 hours compared to a control muffin; however, there were no significant differences between the whole and ground flaxseed muffins. This result can be explained by the fact that whole flaxseed can be effective in lowering blood glucose due to the necessity of chewing the whole seeds, shredding the seed coat and exposing the nutrients inside the seed to digestive enzymes, thus releasing the benefits of the seeds in regard of the glycaemic response (Morris, 2007). Another explanation can be found through reference to the results presented in

Chapter 2 (Section 2.2.2) showing that both forms of flaxseed muffins contained less CHO and starch. To further support this, SDS results (see Section 2.3.3) showed a higher SDS for the ground flaxseed muffins, although there was no significant difference between the two forms of flaxseed muffin as calculated data from the SDS results (see Section 2.3.3) were 12.5 g per muffin for the whole flaxseed muffin, compared to 13 g per muffin for the ground flaxseed.

The results for GR and IR support the short term effectiveness of flaxseed to lower the GR. The results for the blood glucose iAUC in the current study agree with the findings of Dahl *et al.* (2005), who showed that the iAUC in flaxseed fibre bread was significantly lower than the control bread. The blood glucose peak values for the flaxseed were lower than the control; this may be due to the effect of dietary fibre in the flaxseed, which can reduce the gastric emptying time and this can affect the level of postprandial GR (Thakur *et al.*, 2009). Moreover, insoluble dietary fibre in flaxseed has an important role in reducing the GR by slowing the rate of glucose release into the blood (Kapoor *et al.*, 2011). During digestion, dietary fibre is processed using a different pathway to other types of CHO. In the small intestine dietary fibres are not fully digested, which means blood glucose levels are lower after their consumption than after ingestion of other types of CHO (Tappy *et al.*, 1996). This explained finding lower iAUC for blood glucose post consumption for both flaxseed muffins for all time points (Table 4.3). These results are consistent with the results of blood glucose iAUC in Chapter 3 in this thesis (see Section 3.3.3) and all the explanations were fully discussed in Chapter 3 (see Section 3.4).

The results for the GR based on gender showed that there were no significant differences between the genders in the current study, which is consistent with the

study by Wolever *et al.* (2003) that concluded that gender had no effect on the GR results.

The results of the insulin concentration and the change in insulin concentration agree with the results of Kristensen *et al.*, (2013), who found that the dietary fibre in flaxseed is effective at lowering postprandial insulin levels in healthy men. This can be explained by the effect of dietary fibre in the flaxseed which can reduce the release of glucose, which leads to a reduced requirement for insulin from the peripheral tissues (Sonksen and Sonksen 2000). The effects on insulin secretion of flaxseed supplementation could possibly be explained by flaxseed protein stimulating insulin secretion (Nuttall *et al.*, 1984). This is supported by studies that have shown that flaxseed lignan can suppress phospho-enolpyruvate carboxykinase (PEPCK) gene expression, which is involved in glucose production via the gluconeogenesis pathway. Based on this, it is plausible that the lignan found in flaxseed could suppress gluconeogenesis and inhibit glucose production, thereby helping glycaemic control (Prasad 2002; Mohammadi- Sartang *et al.*, 2018). The study by Pan *et al.* (2007) used 360 mg/day of flaxseed-derived lignan supplement for 12 weeks to investigate the effects on T2D patients and found there were statistically significant improvements in glycaemic control, which further supports this hypothesis.

In terms of the results of the iAUC for insulin and insulin peak values for the current study, they disagree with the findings of Au *et al.* (2013) who used glucose solutions containing flaxseed gum (0.7 %). They found that there was no significant difference in the iAUC and time to peak for insulin; the lack of any significant results is possibly due to their small sample size of 12 healthy participants, whereas the current study has a larger sample size of 32 participants. Another difference was that Au *et al.*

(2013) used flaxseed gum in a glucose solution with dairy products while in the current study ground and whole flaxseed were used in muffins as well as the amount of flaxseed which was added to the muffins with 48 % . In the results of Au *et al.* (2013) there was not any observed effect with regard to IR due to using dairy products which may have no effect on IR because of the release of amino acids in the plasma especially leucine, isoleucine, valine, lysine, and threonine (Nilsson, Holstand Björck *et al.*, 2007). Moreover, the level of glucose-dependent insulinotropic polypeptide (GIP) in the plasma was higher post whey meal consumption compared to the reference bread (Nilsson *et al.*, 2004).

The reduction in insulin following the flaxseed muffins may be due to not only the TPC but also the nutritional composition, particularly the fat and protein content (see Section 2.2.2., Table 2.2). Protein has an impact on insulin as it stimulates the secretion of insulin when it is added to oral carbohydrate loads. However, different proteins produce different impacts due to differences in their absorption (Gannon *et al.*, 1988). It is probable that this is caused by the potentiating impact of amino acids on the β -cell. Consequently, it may not be an easy task to draw general conclusions concerning the impact of dietary protein on insulin response. Furthermore, whereas studies on the impact of protein on postprandial insulin responses are of interest physiologically, there is certainly evidence that implies that increasing the protein content of a test meal from 0 to 15 g doubles the insulin secretion (Spiller *et al.*, 1987). That agrees with the current study's insulin results in both flaxseed muffins compared to the control as they had a higher amount of protein (14.8g and 16.8g per serving) for whole and ground respectively (see Section 2.2.2).

The role of fat in insulin production could lie in its delaying gastric emptying, consequently decreasing both the glucose absorption rate and the increase in postprandial insulin (Welch *et al.*, 1987). Nevertheless, of the amount of fat that would need to be added to 50 g of carbohydrate in order to observe such impacts, 30–50 g which would provide 50–65 % of the total energy. The impact of smaller amounts of fat is uncertain; reliable evidence suggests that fat has no impact on postprandial glucose or insulin responses in diabetic people (Gannon *et al.*, 1993). Moreover, it is possible that the impact of fat on gastrointestinal motility in normal persons may be dependent on external elements such as the daily fat intake (Morgan *et al.*, 1988a; 1988b). Consequently, generalisation regarding the impact of dietary fat on postprandial insulin responses may be difficult.

It is apparent from an increasing amount of evidence that a number of polyphenols may have an impact on the carbohydrate metabolism at several levels (Johnston, Clifford and Morgan 2002; Johnston *et al.*, 2005; Törrönen *et al.*, 2009; Hanhineva *et al.*, 2010). Such mechanisms are inclusive of glucose absorption and the inhibition of carbohydrate digestion within the intestine. They also include glucose uptake in the insulin-sensitive tissues and the activation of insulin receptors, as well as gene expression and the modulation of intracellular signalling pathways (Hanhineva *et al.*, 2010). The many reactions involving the secretion of insulin by the pancreas may be possible targets for the action of polyphenols. In the case of blood glucose concentrations being high, pancreatic α -cells respond to the growing demand for insulin through several mechanisms. Such mechanisms include hypertrophy, increased secretion of insulin, and multiplication of existing α -cells leading to the formation of new cells from progenitor ones (Rutter, 2001; 2004).

The long-term impacts of flaxseed muffins on glucose metabolism may indicate that the antioxidant impact of flaxseed can decrease plasma glucose and HbA_{1c} concentrations (Cunnane *et al.*, 1993). A link between the effectiveness of SDG in reducing diabetes development and a decrease in the oxidative stress has been suggested (Prasad, 2002). Moreover, SDG in flax meal is successful in the reduction of diabetes development, and this impact of SDG in diabetes has a connection with a reduction in oxidative stress (Prasad, 2002). Therefore, chronic oxidative stress caused by hyperglycaemia may occupy a significant function in progressive β -cell dysfunction. Consequently, it is possible that enhanced glucose homeostasis will improve pancreatic cell function, sensitivity and insulin secretion (Mani *et al.*, 2011). The hypoglycaemic action of flaxseed powder may be of assistance in averting hyperglycaemia among individuals with diabetes, which subsequently may avert or delay the development of secondary diabetes complications (Mani *et al.*, 2011). Consequently, flaxseed may have a function in decreasing dyslipidemia, obesity and diabetes risk elements (Morisset *et al.*, 2009).

The results for the AUC for satiety agree with Willis *et al.* (2010), who found that there was no significant differences in satiety after consumption of baked products which contained 0-12 g fibre (from pectin, barley beta glucan and guar). Previous studies have suggested that flaxseed consumed in a drink is more effective with regard to satiety than flaxseed in baked products due to the relative digestibility of the fibre in the drinks compared to baked products (Lyly *et al.*, 2010; Mattes and Rothacker, 2001; Vuksan *et al.*, 2009). Studies have suggested that the viscosity of fibres may be greater when given in a liquid form, compared to a solid meal where they may cause only a slight rise in chyme viscosity and this means their effects may be masked by the satiety effect of the solid meal itself (Hoad *et al.*, 2004; Lyly *et al.*,

2010). Dietary fibre viscosity, especially polysaccharides in the flaxseed, can form a gel in the stomach which may lead to a lower gastric emptying time as the gel formation can cause feelings of fullness for a longer time post consumption (Dhingra *et al.*, 2012). Moreover, because fibre cannot digest in the small intestine it provides a bulk which increases satiety (French and Read, 1994). However, the actual mechanisms by which viscous fibre can increase satiety have not been fully elucidated, but it has been suggested that there is some oral influence as a viscous texture in the mouth could help to induce early fullness (Marciani *et al.*, 2001).

Further evidence for the increase in satiety comes from the work of Ibrügger *et al.* (2012) who conducted two studies; the first was to compare the effect of flaxseed drinks and control drinks on satiety, and the second study that compared the effect of flaxseed drinks and flaxseed tablets on satiety. The results of the first study support the theory that a flaxseed drink has a higher AUC for satiety while the results of the second study showed there were no significant differences between flaxseed in different forms (drinks and tablets). The differences may be due to the ability of the flaxseed tablets to dissolve in the stomach and raise the gastric chyme viscosity, which may lead to a decrease in appetite (Marciani *et al.*, 2001). The second study of Ibrügger *et al.* (2012) which compared the effect of flaxseed drinks and flaxseed tablets on satiety had the same trend as the results of the current study, as there were no significant differences between the two flaxseed muffin types, which were both higher than the control.

The absence of any impact of flaxseed on satiety is consistent with some studies that did not find any significant difference in satiety results comparing low, medium or high GI breakfasts (Mahoney *et al.*, 2005; Smith and Foster, 2008). These are

consistent with the outcome of this study, since flaxseed muffins have a low GI, whereas the GI of the control muffins was medium (as shown in Chapter 3). These results may also be interpreted with regard to the category scales that were applied, together with the inter-individual variations and restrictions (Raben, Tagliabue and Astrup, 1995).

The use of category scales works along the same principles as Visual analogue scale (VAS); there is self-reporting by the subjects of their feelings of satiety or hunger in response to specific questions (Jeon, O'Mahony and Kim, 2004). Category scales, are usually nine-point hedonic scales which are labelled using verbal phrases and the subjects choose one of these categories with instructions about how to make their judgments on the scale (Schutz and Cardello, 2001). VAS scales on the other hand are considered to be indeterminate due to the lack of instructions for the subject about how to make their judgments, as they can make their response on any point on the line and there is no category for them (Solah *et al.*, 2015). That means that the VAS is more sensitive, precise and reliable for measurements for the satiety compared to the categorised scale (Schutz and Cardello, 2001; Solah *et al.*, 2015). This indicates that the category scale applied in the current study was only basic compared to a VAS scale. In the current study only a single sensation (hunger) was assessed, consequently failing to show the other parameters such as, fullness, prospective consumption, and desire to eat.

Although there has been a development of techniques to evaluate subjective appetite feelings, upon being questioned, subjects are compelled to consider feelings which usually function subconsciously; this probably has an impact on their reply. However, from another perspective, food intake (for example, *ad libitum*) may provide a more

objective appetite assessment (Jakobsen, 2015). The *ad libitum* test meal can be regarded as an applied method of evaluating a preload's effect on appetite and energy intake. The test is also able to provide an indication of the satiating aspects of the entire diet. This enables the direct monitoring of patient's eating patterns by recording the foods which are eaten and also applying nutritional analysis software in order to calculate nutrient and energy intake (Benelam, 2009). Unfortunately, this was not able to be completed within the methodology for the current study.

The results for the sensory evaluation of the flaxseed muffins was similar to those of the control muffins. Other studies on the acceptance of flaxseed products, such as Ramcharitar *et al.* (2005), had different results; here participants rated the control more highly than the flaxseed muffins ($p < 0.001$) and all sensory attributes for the added flaxseed were low. Hussain *et al.* (2006) found that participants did not rate flaxseed biscuits containing 25 % and 30 % flaxseed very highly compared to the control biscuits. The high acceptance in the current study can be explained by the addition of ground cinnamon and nutmeg as well as banana which gave a more acceptable flavour as flaxseed alone may be considered to have a nutty flavour which may not always be acceptable (Przygodzka *et al.*, 2016). Yeu ,Lee and Lee (2007) found that adding cinnamon enhanced the taste, the aroma and overall acceptance of a soy based, high protein breakfast cereal. This is in agreement with the results of Khouryieh and Aramouni (2013) who found that 12 % flaxseed cereal bars were highly acceptable to participants due to the added cinnamon in the formulation.

There are differences in terms of sensory evaluation between the different forms of flaxseed (whole, ground or flour) added to bakery products. These differences can be explained by the variations in their nutrient content, such as the amount of the fat and

fibre found in each form and this depends on the way the seed was processed. The processing method will produce different consistencies and textures, which affect the overall acceptance of the muffins produced (Daun *et al.*, 2003). According to the USDA (2017) and Hussain *et al.* (2008), ground flaxseed contained 42.86 g/100 g of fat, while whole flaxseed flour contained 37 g/100 g of fat; whole flaxseed flour contained 8.2 g/100 g of fibre, while ground flaxseed contained 21.4 g/100 g of fibre.

Lipilina and Ganji (2009) showed that replacing flour with 50 % of ground flaxseed in a muffin and 30 % in bread had the most acceptable sensory evaluation compared to control bread with wheat flour. Ground flaxseed was the best option to add to bakery products as it enhanced the sensory attributes and could be added in high amounts (up to 50 %) without any negative effects (Alpers and Sawyer- Mores 1996). The hull in the flaxseed has a higher oil content resulting in an oily texture when using milled or crushed or whole flaxseed (Vaisey-Genser and Morris, 1997).

Whole flaxseed gives a crunchy texture to bakery products due to the presence of soluble fibre, especially mucilage, found in the seed coat (Morris and Vaisey-Gennser 2003; Singh *et al.*, 2011). Ground flaxseed is prepared by crunching and grinding the flaxseed and the process may remove the hard coat and reduce the amount of the mucilage and crude fibre in the final product (Dev and Quensel, 1988).

The results for the evaluation of texture are consistent with those of Hussain *et al.* (2006) and Ganorkar and Jain (2014), who found that flaxseed cookies with different percentages of roasted flaxseed flour as replacement for wheat flour had a lower rating for texture compared to the control. In these studies the texture was more acceptable when flaxseed was added in small amounts, such as 5 %. The difference

between these studies and the current study was the flaxseed form (ground vs. whole, full fat flaxseed flour and roasted flaxseed flour), which might account for the differences in acceptability. Although the amount of flaxseed in the current study was higher than the other studies, the acceptance of the texture was still high. The level of acceptability of the muffins may be explained by the addition of mashed banana, which enhances the textural acceptance in the muffins (Rudrawar and Jadhav 2015).

The results for the gastrointestinal symptoms indicated that both types of flaxseed (whole and ground) produced some side effects on the gastrointestinal system; they principally caused diarrhoea, flatulence, belching and abdominal pain, but this was not in a high number of participants. These results are consistent with the results of Pruthi *et al.* (2012), who examined the effect of adding flaxseed to bar among postmenopausal women, on the gastrointestinal symptoms compared to the placebo over a period of 6 weeks. They found that flaxseed had an effect, with the occurrence of some gastrointestinal symptoms such as abdominal distention, flatulence and diarrhoea. There were no significant differences in those symptoms compared to the placebo, which disagrees with the current study and that can be referred to the differences between the study groups (healthy male and females vs. postmenopausal women), the test foods (flaxseed bar vs. muffins) and the amount of flaxseed (7.5g fibre for the bar vs. 30g for the both flaxseed muffins). These results can be explained by the presence of soluble fibre, mainly in the form of mucilage, which forms a gel-like substance on contact with water and this may contribute to stool softening, while the insoluble fibre can be responsible for the faecal bulking effect observed with flaxseed use (Cockerell *et al.*, 2012).

Satiety was measured in the current study using a categorised scale (see Section 4.3.6) and the GI symptoms questionnaire also included a question on fullness (see Section 4.3.8). The 2-hour satiety results were not significant for the flaxseed muffins compared to the control muffins (see Section 4.3.6), while the postprandial fullness of the GI symptoms 24 post consumption (see Section 4.3.8) were significantly higher for both flaxseed muffin types compared to the control muffins. This may indicate that in short-term research studies, the connection between discerned hunger and food intake has a considerable dependence on the time at which satiety is assessed. This could be applicable to the present study because the satiety measurement was applied for only two hours. However, the questionnaire on GI symptoms included a question on fullness carried out 24 hours post consumption, which was high for the flaxseed muffins in comparison to the control muffins. This may be because fibre has a long-term impact on satiety.

Burley *et al.* (1987) found that that a high-fibre breakfast resulted in greater fullness in the appetite ratings of participants. The fibre amount was significant, and larger doses had a greater impact in advancing satiety and decreasing energy intake. High fibre doses (such as over 10 g in one dose or 30 g during the whole day) showed improved satiety results (Benelam, 2009). According to Slavin and Green (2007), food digestion and nutrient absorption could be extended by viscous fibres, thereby increasing the time available for the induction of pre-absorptive and post-absorptive satiety mechanisms. Chewing time and gastric distension can be increased by the bulking impact of fibre, thereby advancing satiation. Furthermore, the addition of fibre decreases energy density which may also induce satiety (Slavin and Green, 2007).

4.5 Summary

Overall, the results of the current study have highlighted the importance of investigating the addition of flaxseed in different forms to bakery products, in terms of GR and IR. Most previous studies have used ground flaxseed, as it has been suggested that whole flaxseed is more likely to pass through the digestive system without releasing the full benefits of the seeds compared to ground flaxseed which may be easier to digest and therefore provide more health benefits (Morris, 2007). The results for this study did not support this hypothesis as whole flaxseed muffins had an effect of lowering GR/IR compared to the control muffins. All results for GR/IR for whole flaxseed was significantly lower than the control muffins. The results for the whole flaxseed muffins were similar to those from ground flaxseed muffins.

The current study focused on comparing the effects of the addition of whole or ground flaxseed in muffins, investigating changes in GR and IR. In this study the results showed that muffins containing flaxseeds in different forms, either whole or ground, both had the effect of lowering GR/IR compared to control muffins. There were no significant differences between either forms of flaxseed muffin; moreover, the flaxseed in the whole form was as effective as the ground flaxseed in terms of GR, IR and satiety.

As hypothesized, flaxseed had an effect on the glucose and insulin levels, which was the starting point for this study. The main results from this study was that muffins containing flaxseed in whole and ground form both produced lower GR and IR compared to the control muffin. However, the sensory evaluation results indicated greater acceptability for the ground flaxseed muffins than the whole flaxseed muffins.

That was the rationale for using the ground flaxseed for the next study which aimed to investigate the most effective portion regimen of flaxseed, whether flaxseed muffins are more effective when given once or more than once during the day, on the 24 hours blood glucose among healthy participants using continuous glucose monitoring system.

Chapter 5 : Effect of a split portion of flaxseed on 24-hour on glucose response

5.1 Justification and aims

CHO in low GI foods takes a longer time to be absorbed compared to high GI and this results in lower glucose peak values, which can help to stabilise the blood glucose at a normal level for longer time (Willett , Manson and Liu ., 2002).

Different studies have all agreed that the consumption of low GI foods can minimize the potentiality large fluctuations in blood glucose levels (Brand-Miller *et al.*, 2003; Kaur *et al.*, 2016). Blood glucose levels fluctuate widely during the postprandial time which leads to greater oxidative stress, which could ultimately cause impaired glucose tolerance and T2D (Monnier *et al.*, 2006).

Foods which contain a high amount of fibre generally have a lower postprandial blood glucose and insulin response and this can help to prevent T2D in healthy individuals (Riccardi ,Rivellese and Giacco 2008). Flaxseed is considered to be one of the key plant sources of dietary fibre, as it contains soluble and insoluble fibre and it considered having a low glycaemic CHO (Tarpila *et al.*, 2005; Singh *et al.*, 2011).

An individual's plasma glucose values can fluctuate depending on the state they are in; if they are postprandial, post-absorptive, or fasting the values will be different (Brynes *et al.*, 2005). Current eating patterns can mean that a high proportion of the day is spent in the postprandial state, which produces the highest plasma glucose levels (de Vegt *et al.*, 1999). Studies have suggested that high postprandial glycaemic responses can be metabolically disadvantageous and therefore life-style changes such as diets that are specifically designed to reduce postprandial glycaemia may be

beneficial as they could provide some protection against CVD (de Vegt *et al.*, 1999; Brynes *et al.*, 2005).

Previous studies have demonstrated that flaxseed can be effective at lowering and stabilising the blood glucose response in healthy, pre-diabetic and diabetic individuals (Cunnane *et al.*, 1995; Thakur *et al.*, 2009 and Hutchins *et al.*, 2013). The question remains as to whether flaxseed is more effective at maintaining blood glucose at the normal level over 24 hours when the portion is given once or more than once during the day. Since the majority of studies gathered the data in the laboratory, this may not represent the real situation that occurs over a full day.

The MiniMed CGMS supplies a method of evaluating subcutaneous interstitial fluid glucose over a period of three days, by applying an electrochemical detector under free-living conditions (Mastrototaro, 1999). CGMS evaluates interstitial glucose concentrations every five minutes and are minimally invasive, which gives an opportunity to evaluate the many temporal changes in blood glucose level (Phillip *et al.*, 2012)

For that reason, it was decided in this study to use the CGMS, as it provides information about the direction, magnitude, duration, frequency and causes of fluctuations in blood glucose levels (Mastrototaro, 1999). It can give a detailed view of the blood glucose profile, which can potentially show when and to what extent, an intervention affects the GR, while allowing the participants to continue their daily lives and activities uninterrupted (Henry *et al.*, 2006; Kaur *et al.*, 2016).

Currently, to the best of our knowledge, no study has investigated the effect of splitting the portion of flaxseed on blood glucose control. The hypoglycaemic effect

of the flaxseed, as demonstrated by Cunnane *et al.* (1995), Thakur *et al.* (2009) and Hutchins *et al.* (2013), would be enough to lower and maintain the blood glucose whether the portion of flaxseed was administered in a single portion or in split portions. However, it could be more effective if the portion were split over the three daily meals so that its effect was spread over time, which could cause a lower blood glucose measurement after each of the three meals compared to the single portion. This could minimise the potentially large fluctuations in blood glucose levels during the day to avoid the occurrence of hyper- and hypo-glycaemia.

The aim of this study, therefore, was to determine the most effective portion regimen among healthy participants. This study used a single and a split-portion regimen and investigated the blood glucose response over a 24-hour period using a CGMS to determine whether flaxseed was more effective when consumed as a single portion of 30g or as a split portion consumed three times per day (10 g flaxseed per portion). In this study ground flaxseed muffins was used as the results from *in vitro* results (Chapter 2) that it was higher on TPC and antioxidants, for the results of GI (Chapter 3) it had a lower GI compared to the whole flaxseed muffins.

5.2 Methods

The methods for this study were based on two similar studies carried out within the Oxford Brookes Centre for Nutrition and Health by Henry *et al.* (2006) and Henry, Newens and Lightowler (2009) which used Medtronic MiniMed (Northridge, CA, USA) 24-hour blood glucose sensors with healthy participants. The current study used a split-portion of ground flaxseed muffins compared to control muffins and investigated the blood glucose response over a 24-hour period using CGMS. Ethical

approval for the study was obtained from UREC at Oxford Brookes University (UREC Registration No: 161046) (Appendix 4.a).

5.2.1 Participants

Healthy male and female adults aged between 18-60 years were recruited to this study at Oxford Brookes University through advertisements with the researcher contact details. These were placed on the notice boards in different departments at Oxford Brookes University, on the volunteers' section of a local website (<http://www.dailyinfo.co.uk/>) and there was also informal recruitment through personal communications between March 2017 to November 2017.

Participants interested in taking part in the study were provided with a detailed PIS (Appendix 4.b) to keep; it contained all the information needed to take part in the study, as well as the preparation required for all visits. The inclusion criteria were similar to those for the study of determination of GI in Chapter 3 (see Section 3.2.1).

5.2.2. Study design

The study was a randomised, cross over repeated-measures design. Randomisation of the order of the participants' split-portion regimen was carried out using a random number generator website (<https://www.randomizer.org>). This study involved seven visits (Figure 5.1); the first was the screening visit, the six test visits were divided into three main sessions and three follow-up sessions. At the screening visit, participants were provided with the PIS to keep and they were encouraged to ask questions regarding taking part in the study. If they agreed to take part they then were asked to

sign a consent form (Appendix 4.c). All the requirements for the screening visits were similar to in the study of determination of GI in Chapter 3 (see Section 3.2.2).

On the main sessions CGMS sensor was inserted under the skin in the abdominal region between 15.00-17.00h. Participants also collected the pre-prepared food for an evening meal for the day before the test day, as well as food for each test day, to ensure that all participants began the test day following a similar meal. There was no need to fast for the sensor insertion session and there were no restrictions on the types of clothes that they could wear.

Participants were required to remain at the Oxford Brookes Centre for Nutrition and Health for 1 hour to allow the sensor to initialise after trained personnel inserted the sensor. The participants were required to take four capillary finger-prick blood glucose readings using a single use lancing system (Unistik 3, Owen Munford, Woodstock, UK). The readings needed to be taken at the following times, one and two hours after the insertion of the sensor, before dinner and before bedtime. The purpose of the capillary finger-pricks was to calibrate the CGMS sensor in order to record the blood glucose readings during insertion.

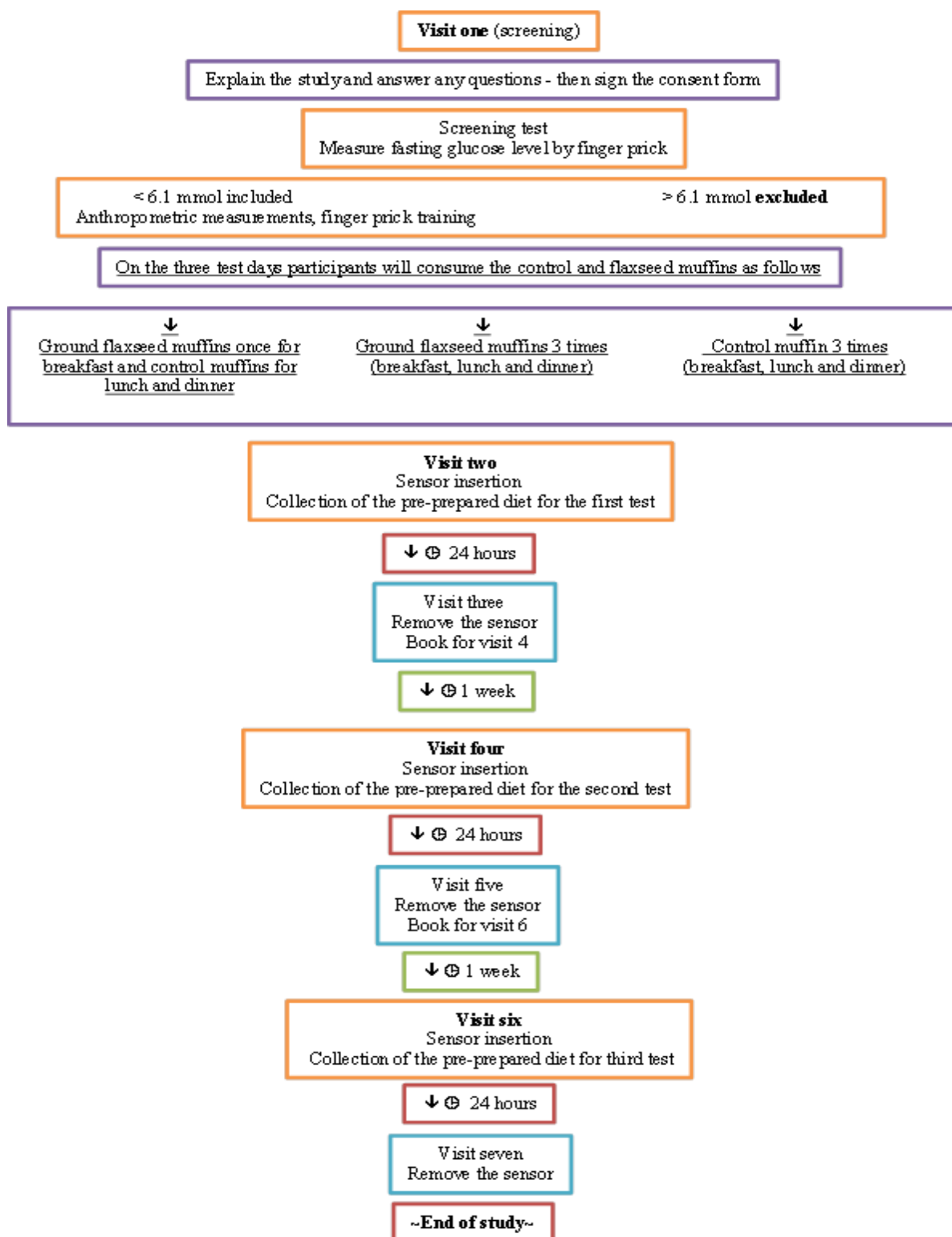


Figure 5.1. Summary of the CGMS study protocol.

All participants were provided with a blood glucose meter device (ACCU-CHEK® Performa Nano, Roche Diabetes Care Limited, Charles Avenue, Burgess Hill UK). Participants were provided with written instructions on using the device and

performing a capillary finger-prick; all participants were also trained to carry on taking blood glucose readings by themselves. While the sensor was inserted, participants were required to avoid alcohol and restrict intense physical activity (e.g. long periods at the gym, running, aerobics). Dinner on that day needed to be consumed between 18.00-20.00 h.

On test day 1 which came the day after sensor insertion, the participants were required to take four capillary finger-pricks to measure blood glucose readings following the same procedure as the day prior the test day (before each main meal and before bedtime). Participants were aware of how to follow the correct procedures to dispose of the waste safely and they were provided with a clinical waste bin to dispose of all the items after use.

The participants were instructed about when to take their meals on the test day; breakfast between 7.00 - 9.00 h, the first snack between 10.00-11.00 h, lunch between 12.00-14.00 h, the second snack between 16.00-17.00 h and dinner between 18.00-20.00 h. They were told not to consume any food or drink which was not provided, apart from water and hot drinks such as tea, coffee or herbal tea. However, they needed to record all the drinks consumed.

On the three test days: participants randomly consumed either control or flaxseed muffins in one of the following orders:

- Test one – flaxseed once a day: 3 ground flaxseed muffins for breakfast, one control muffin for lunch and dinner.
- Test two – flaxseed 3 times a day: one ground flaxseed muffin and 2 control muffins at breakfast and one flaxseed muffin at lunch and dinner.

- Test three – control muffins 3 times a day: 3 control muffins at breakfast and one control muffin at lunch and dinner).

The order and number of muffins (control, flaxseed) for each test day are shown in Table 5.1.

Table 5.1 Order and number of muffins (control, flaxseed) for each test day

	Control	Flaxseed once	Control Flaxseed three times	Flaxseed once	Flaxseed times
				Flaxseed once	Flaxseed times
Breakfast		Breakfast 			
		Breakfast 			
Lunch		Lunch 			
Dinner		Dinner 			
		Dinner			
		=Flaxseed muffin		=Control muffin	

On the follow up sessions the participants attended the Oxford Brookes Centre for Nutrition and Health in the morning between 7.00-9.00 h after an overnight fast for the CGMS sensor removal. One final capillary finger-prick for glucose reading was taken to calibrate the sensors before removing. There was a washout of at least one week between the main test sessions. The wash out period was needed to minimise the carry over effect between the different treatments. One week as wash out was based on studies by Brynes *et al.* (2005) and Morgan *et al.*,(2012).

5.2.3 CGMS sensor



Figure 5.2. CGMS sensor the Medtronic MiniMed iPro®2.

Measurement of the 24-hour glucose response was made using the Medtronic MiniMed iPro®2 Professional Continuous Glucose Monitoring (Northridge, CA, USA; Figure 5.2) which recorded glucose levels every 5 min, giving a total of 288 readings over 24 hours.

The CGMS is a technology that has been previously used in other studies Henry *et al.*, (2006) and Henry, Newens and Lightowler (2009). This technology provides a full view of the glycaemic level every 5 minutes, to potentially show when and to what

extent, an intervention affects the glycaemic response. It also allows the participants to continue their daily lives and activities uninterrupted (Henry *et al.*, 2006). O'Riordan *et al.* (2009) found that the CGMS performance on two occasions over a 12-month period were reliable, reproducible and repeatable.

Using an applicator or self-insertion device, a thin plastic sensor is inserted just under the skin of the abdomen by trained personnel. The receiver can store information for later use and long-term data can be downloaded to a computer programme CareLink iPro Software, which is a Web-based system, designed to generate reports and store data (Kaufman *et al.*, 2001; Hanaire, 2006 and Burge *et al.*, 2008). The CGMS has been used safely in many studies among patients with type 1 and T2D (Chico *et al.*, 2003) and among children with type 1 diabetes (Wilson *et al.*, 2006).

5.2.4 Muffins

The muffins (flaxseed and control) were prepared using a standard recipe (see Section 2.2.1) with 10 g of flaxseed added per muffin for the flaxseed muffins and no added flaxseed for the control muffins (Table 5.2).

Table 5.2. Muffin formulation(g) for twelve muffins

Ingredients	Muffin formulation		
	Unit	Control	Ground flaxseed
Melted butter	g	75	75
Self-rising flour	g	250	130
Flaxseed	g	-	120
Baking powder	g	5	5
Bicarbonate of soda	g	2.5	2.5
Ground cinnamon	g	2.5	2.5
Nutmeg	g	2.5	2.5
Caster sugar	g	115	115
Vanilla extract	ml	5	5
Large ripe bananas	g	240	240
Medium eggs	g	106	106
Semi skimmed milk	ml	125	125

5.2.5 Dietary intervention

The participants were provided with pre-packaged meals for the evening meal and all meals for the test days. The meals were designed to meet the DRVs for energy and macronutrients for males and females based on the recommendations of the Scientific Advisory Committee on Nutrition (SACN, 2012). The meals and snacks were made up from control or flaxseed muffins, orange juice, Greek style yoghurt, apple, hummus sandwich, green salad, vegetable lasagne, low fat berry medley yoghurt, nuts, orange, grapes and an oat and honey bar. Each food was labelled with the time that it should be consumed, as well as the meal type.

All meals were planned using Nutritics software ver 5.022 (Nutritics LTD, Dublin, Ireland) based on SACN (2012) for a healthy standard adult males and females; if the meal components were not on the Nutritics database, all the energy and macronutrient information which was needed to plan the meals for each test day was inserted

manually according to the nutritional information label on that product. (Table 5.3, Table 5.4 and Figure 5.3). For a detailed diet composition analysis of a full day meal see (Appendix 4.d).

Table 5.3 An example diet for dinner prior to the test day and full meals for the test day (based on taking flaxseed muffins once a day for a female participant)*

On day before the test day	Food	Amount
Dinner	Vegetable lasagne	255 g
	Citrus orange fruit	70 g
	Tesco Low Fat Berry Medley Yoghurt	125 g
On the test day	Food	Amount
Breakfast	3 flaxseed muffins	228 g
	Tropicana orange juice	250 ml
	Tesco Fat Free Greek Style Yoghurt	100 g
Snack 1	Nature Valley Crunchy Bars Oat and Honey	21 g
Lunch	Hummus, sandwiches	300 g
	Apples	152 g
	Control muffin	76 g
	Mixed nuts	10 g
	Tesco Simple Salad	135 g
Snack 2	Grapes	35 g
Dinner	Vegetable lasagne	255 g
	Control Muffin	76 g
	Citrus orange fruit	70 g
	Tesco Low Fat Berry Medley Yoghurt	125 g

* The diet was similar for each test days for each participant only the muffins changed between the test days.



Figure 5.3. An example for the meals (top) provided for males and females (bottom).

Table 5.4 The total macronutrients and fibre in each test day meals.

	Control muffins	Once a day flaxseed muffins	Three times a day flaxseed muffins
Males			
Energy (kcal)	2709.5	2711	2709.6
Protein (g)	66.5	67.4	68.7
Carbohydrates (g)	369.6	353.3	356.1
Fat (g)	100.5	106.6	108.3
Fibre (g)	36.2	44.2	44.2
Females			
Energy (kcal)	222.9	2220.2	2224.5
Protein (g)	57.4	63.8	59.9
Carbohydrates (g)	346.3	332.7	328.1
Fat (g)	78.7	80.7	85.9
Fibre (g)	32.4	39.8	38.9

5.2.6 Instructions to Participants

Participants were provided with instructions about the use and care of the CGMS sensors (Appendix 4.e). These instructions were based on the manual from the sensors provided by the manufacturer as well as details on their website. Participants were aware that they could contact the researcher by mobile phone if they had any problems with the sensor. In case of an emergency, they could call the helpline of the sensor company, which offered twenty-four hour support.

Participants were also provided with a blood glucose log sheet to record their blood glucose level measured on the blood glucose meter device provided (Appendix 4.f). They needed to record three measurements for the day prior to the test day; four

measurements for the test day and once on the day of sensor removal session (see Study design. All blood glucose measurements needed to calibrate the CGMS sensor. They need to record the time and the blood glucose level for each measurement. All instructions were on the form as well as being explained to the participants on each main visit.

For the diet, the dinner provided the day before as well as all meals on the test days, there were instructions about the diet and information participants needed to fill in (Appendix 4.g). The instructions listed all the food which was provided together with the time at which each meal should be eaten. Participants needed to fill in the exact time they ate as well as if there were any leftovers; they needed to record any hot drinks such as tea, coffee or other hot drinks they consumed; they needed to include any additions to the drinks, such as sugar, milk or alternatives to them. Participants were encouraged to repeat the consumption of these drinks on the next two test days to in order to standardise the drinks consumed.

Participants also needed to record their physical activity (description of activity type and intensity of the activity) (Appendix 4.h). Participants needed to record this for the day prior to the test day, as well as on the test day to make sure they were following the instructions and restricting intense physical activity (e.g. long periods at the gym, running, aerobics). As the intense physical activity may affect the site of sensor placement during resistance exercise, it is important to make sure the sensor is not located near working muscles, as they can decrease the interstitium glucose supply when longer-duration exercise is undertaken and there is an increased potential for dehydration which leads to decreased glucose levels (Moser *et al.*, 2016, Bally *et al.*, 2016; Moser Yardley, and Bracken *et al.*, 2018).

5.2.7 Analysis of CGMS data

All data from the sensors were downloaded to the CareLink iPro Software for each participant, which was coded with the participant's number and the test number; all capillary results for blood glucose levels were inserted manually for each test treatment. All the times that the meals were consumed were inserted manually and all the data was then exported to Microsoft Excel (Windows version 2010) to complete the data analysis. One day (24 hour data with 288 glucose readings) was determined to be from 06:00 on day 1 to 06:00 on day 2.

For each test day, the following measurements were performed:

- Mean blood glucose concentration for 24 h, day (06.00–22.00 h), night (22.00–06.00 h) and pre - post each main meal.
- The blood glucose iAUC) calculated geometrically for each participant's blood glucose response; the 06.00 h glucose result was taken as the baseline.
- Blood glucose peak values for each main meal, as well as the time to peak.
- Standard deviation calculated for all above measurements, except for mean blood glucose concentration over 24 hours, day and night, where standard error was calculated.

5.2.8 Gastrointestinal symptoms

A gastrointestinal symptoms questionnaire was provided to be filled in for 24 hours after each test day (Appendix 3i). To determine whether participants were having any gastrointestinal symptoms, a form consisting of 100 mm VAS with words anchored at each end was used to assess four different gastrointestinal symptoms. Participants

rated the frequency and the severity of those symptoms. The scales were used to assess frequency (100 mm = ‘much more often than normal to 0 mm = ‘not more often than normal) and severity (100 mm = ‘very severe to 0 mm = ‘none’) of the symptoms (Kristensen *et al.*, 2017).

5.2.9 Statistical analysis

The required sample size for this study was estimated to be 15 to be able to detect a 5 % difference between 2 days in the overall blood glucose iAUC with the confidence level as 95 % and for a medium effect size, with α -0.05 (two-tailed) using G*power (Version 3.0.10). The power of study was 90 % and the number of participants was based on the previous studies by Imai *et al.* (2017) and Kajiyama *et al.* (2018).

Statistical analysis was performed using SPSS software, (Statistical Package for the Social Sciences) version 25, (Chicago, USA). Prior to statistical analysis, the normality of the data was assured using the Shapiro - Wilks statistical test to investigate the normality of the data; where data were not normally distributed, non-parametric tests were used.

All results were reported as the mean $\pm$ SD or SEM. Differences in mean blood glucose concentration, time to peak, iAUC and gastrointestinal symptoms between the three test days were assessed using repeated measures ANOVA followed by a Bonferroni for multiple comparisons if the data was parametric, or the Friedman test and Wilcoxon for non-parametric data. $p < 0.05$ was considered statistically significant.

The Pearson correlation coefficient and the method of Bland and Altman (1986) were used to assess the correlation and agreement between the CGMS sensor data and ACCU-CHEK® data.

5.3 Results

5.3.1 Participants characteristics

Fifteen healthy adults (10 females, 5 males) were recruited and the baseline of the participants' anthropometric measurements (Table 5.1)

Table 5.5. Baseline characteristics for fifteen participants (10 female, 5 male) (mean $\pm$ SD).

	All (<i>n</i> 15)
Age (y)	30.3 $\pm$ 9.1
Height (m)	1.7 $\pm$ 0.1
Weight (kg)	69.7 $\pm$ 13.3
BMI (kg/m ²)	24.1 $\pm$ 2.9
Body fat %	25 $\pm$ 9.3
Fasting blood glucose (mmol/l)	5.0 $\pm$ 0.3

5.3.2 Correlation between the CGMS and the capillary blood glucose readings

The results (Figure 5.4) showed there was a strong correlation observed between the CGMS and the capillary blood finger-pricks carried out using Accu-Chek for the 405-paried glucose readings ($r = 0.57$, $p < 0.001$).

The Bland-Altman analysis (Figure 5.4) indicated a good agreement between the CGMS and Accu-Chek for glucose level for 405-paried readings (mean difference 0.1

mmol; 95 % confidence interval -0.166 to -0.034; limits of agreement -1.4 to 1.3 mmol). These results suggest that the data from the CGMS is reliable.

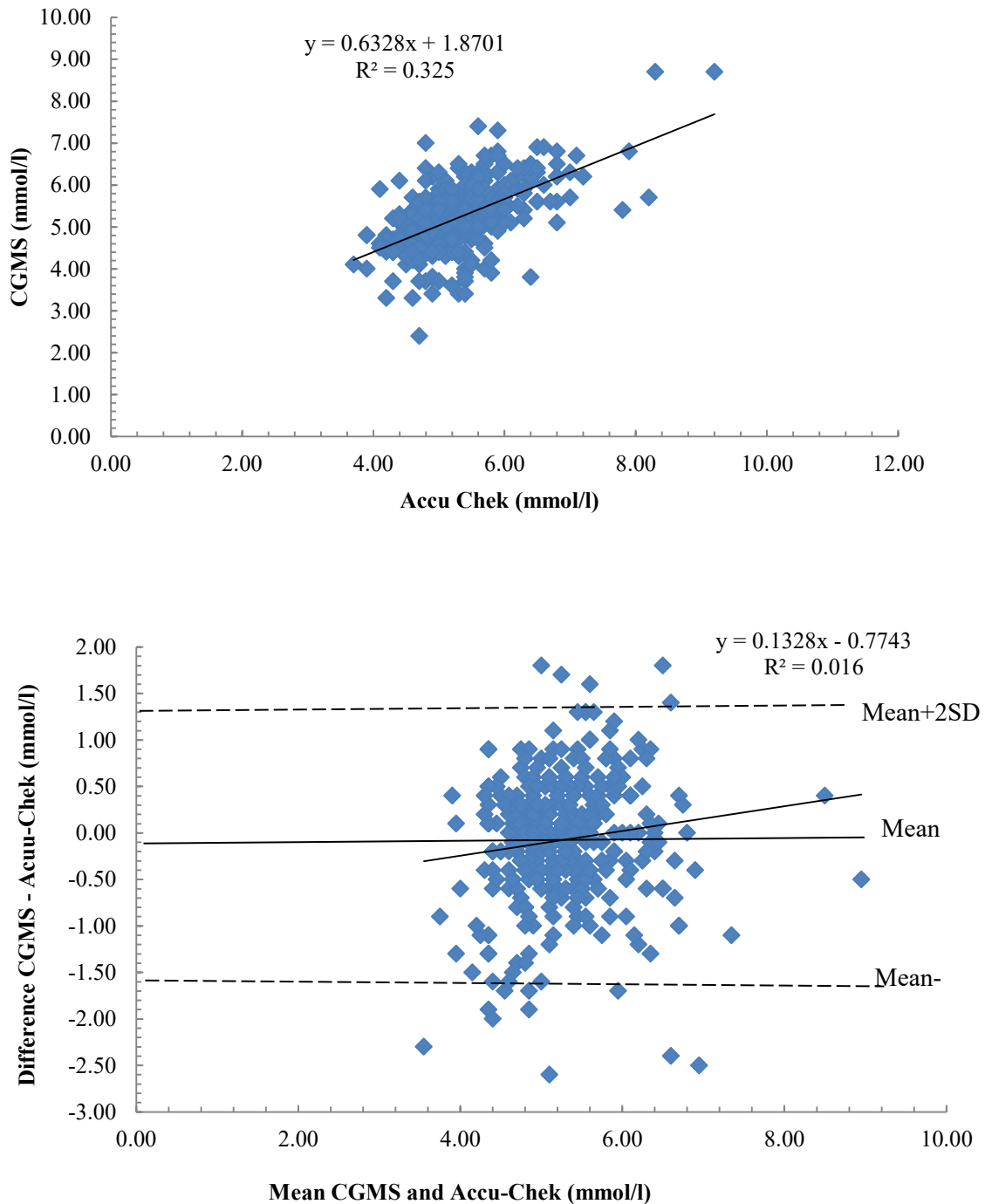


Figure 5.4. Pearson regression of 405 paired glucose measurements between the CGMS™ and Accu-Chek data. Bland Altman analysis of 405 paired glucose measurements between the CGMS™ and Accu-Chek data.

5.3.6 Blood glucose profile

The blood glucose profile over 24 hours for control muffins, once-a-day flaxseed muffins and three-times-a-day flaxseed muffins is presented in (Figure 5.5). The blood glucose profile from breakfast to lunch is presented in (Figure 5.6), from lunch to dinner in (Figure 5.7) and from dinner to the next day is in (Figure 5.8). To standardise the data, the mean change in blood glucose was calculated from the baseline, 6.00 h for the whole period of 24 hours, while for the rest of the investigations (the 6 hours from breakfast, 6 hours from lunch and over 8 hours from dinner) it was calculated as the mean change in blood glucose from the baseline, which was the time of the meal (breakfast, lunch and dinner).

All the investigations showed the same trend: splitting the portion of flaxseed to three times a day produced the lowest results, followed by flaxseed muffins once a day, while the control muffins had the highest glucose levels.

In the 6 hours from breakfast to lunch (Figure 5.6) the flaxseed once treatment produced a lower mean blood glucose compared to the other treatments, especially between 30-120 minutes post consumption, as the whole flaxseed portion of 30 g was consumed at the breakfast.

In the 6 hours from lunch to dinner (Figure 5.7) the results showed that all muffins produced the same peak around 1 hour post consumption with different peak values; the peak values were lower for the flaxseed muffins three-times-a-day compared to the other treatments. All the treatments show a gradual decrease in blood glucose over time; however, the rate of decrease was higher for the flaxseed muffins three-times-a-

day, which produced blood glucose levels lower than the baseline at 210 minutes post consumption, compared to 240 minutes for the other treatments.

In the 8 hours from dinner to the next day (Figure 5.8), the results indicated that splitting the portion of flaxseed to 3 times a day was more effective in lowering blood glucose levels compared to the other treatments, The concentration of blood glucose remained more constant over the time period compared to the other treatments, with a peak value at almost 30 minutes post consumption and it took 210 minutes for the other treatments to reach the 3 times a day treatment levels.

The mean change in blood glucose was calculated from the baseline, 6.00 h for the whole period of 24 hours, while for the rest of the investigations (the 6 hours from breakfast, 6 hours from lunch and over 8 hours from dinner) it was calculated as the mean change in blood glucose from the baseline, which was the time of the meal (breakfast, lunch and dinner).

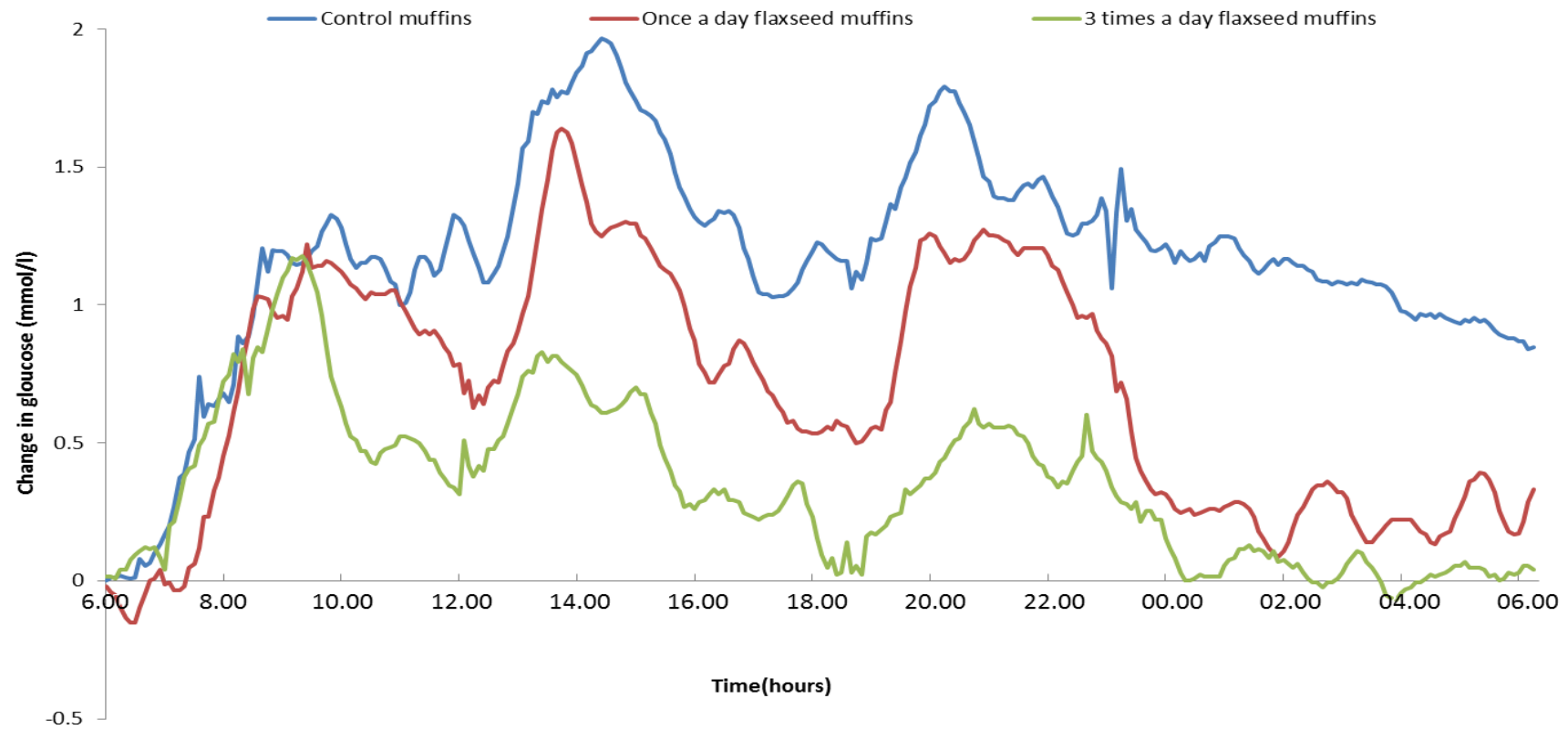


Figure 5.5. Mean change in blood glucose (mmol/l) during 24 h, for each test day from the baseline, which was 6.00 am (n=15)

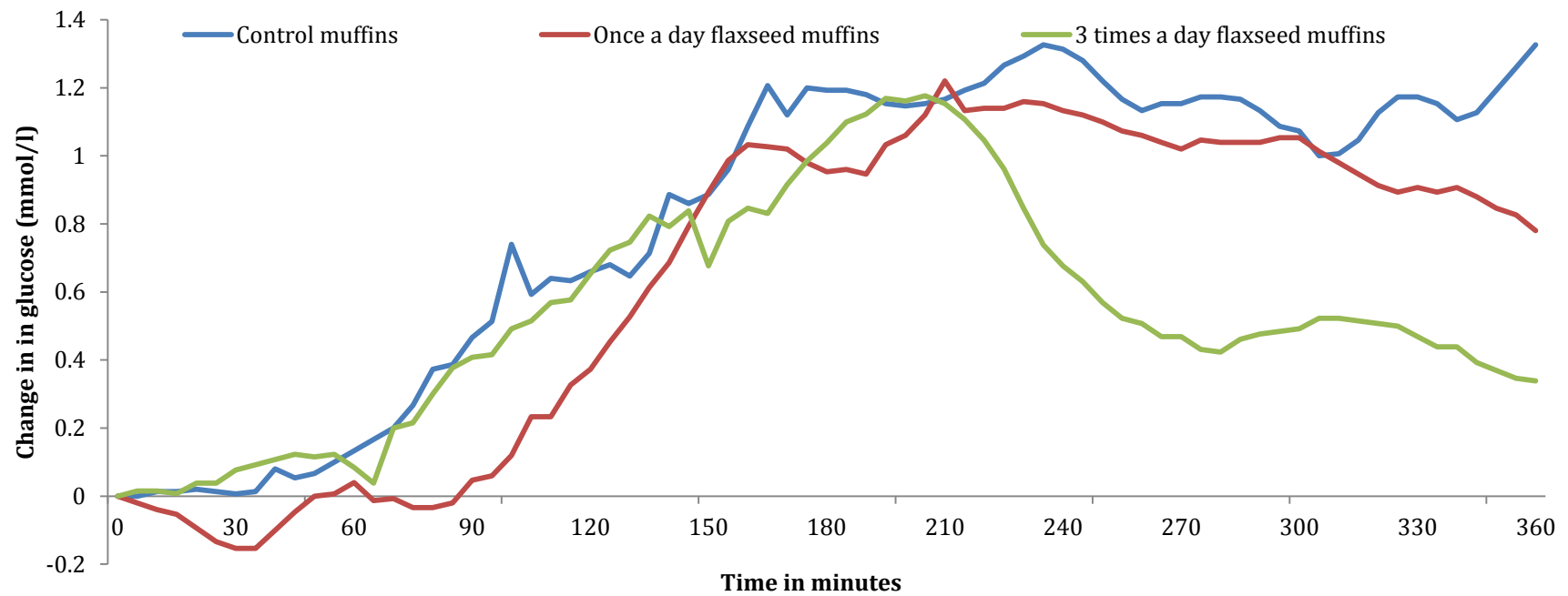


Figure 5.6. Mean change in glucose (mmol/l) 6 hours from breakfast to lunch for each test day calculated as the mean change in blood glucose from the baseline, which was the time of consuming the breakfast (n=15)

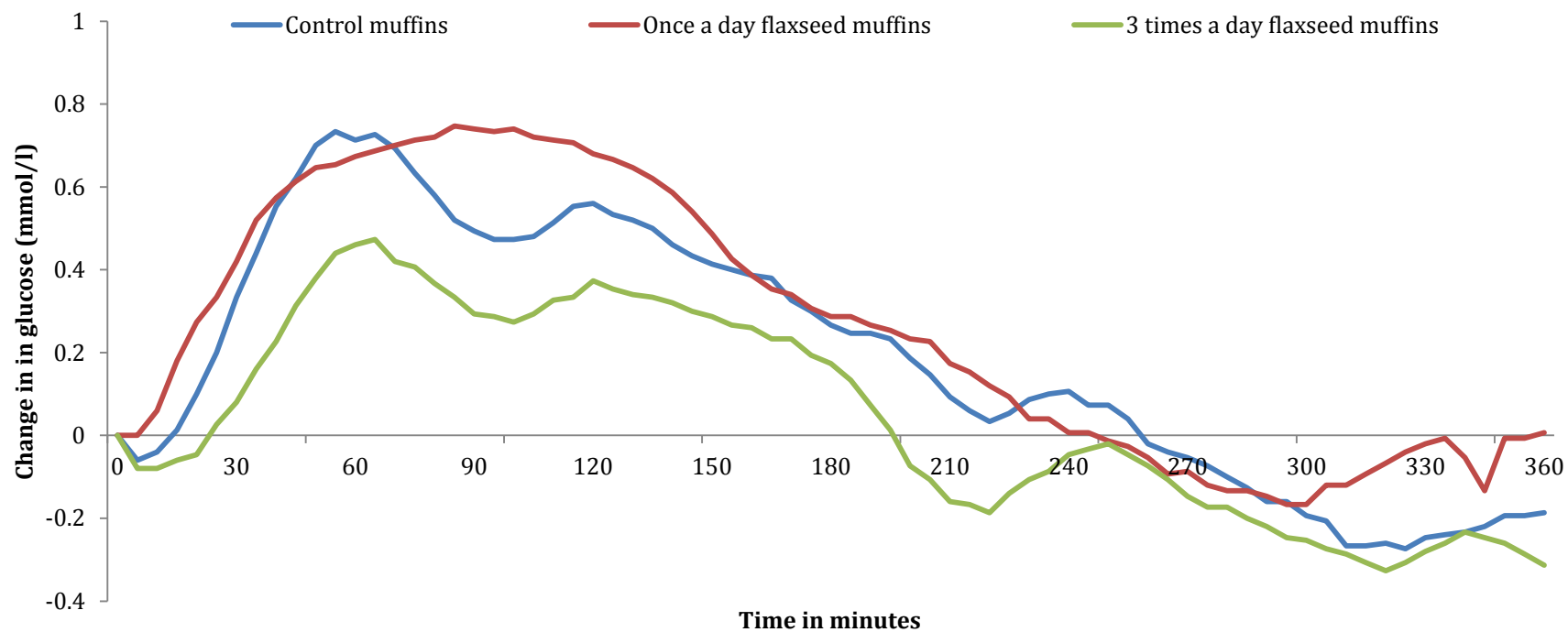


Figure 5.7. Mean change in glucose (mmol/l) 6 hours from lunch to dinner for each test day calculated as the mean change in blood glucose from the baseline, which was the time of consuming the lunch (n=15)

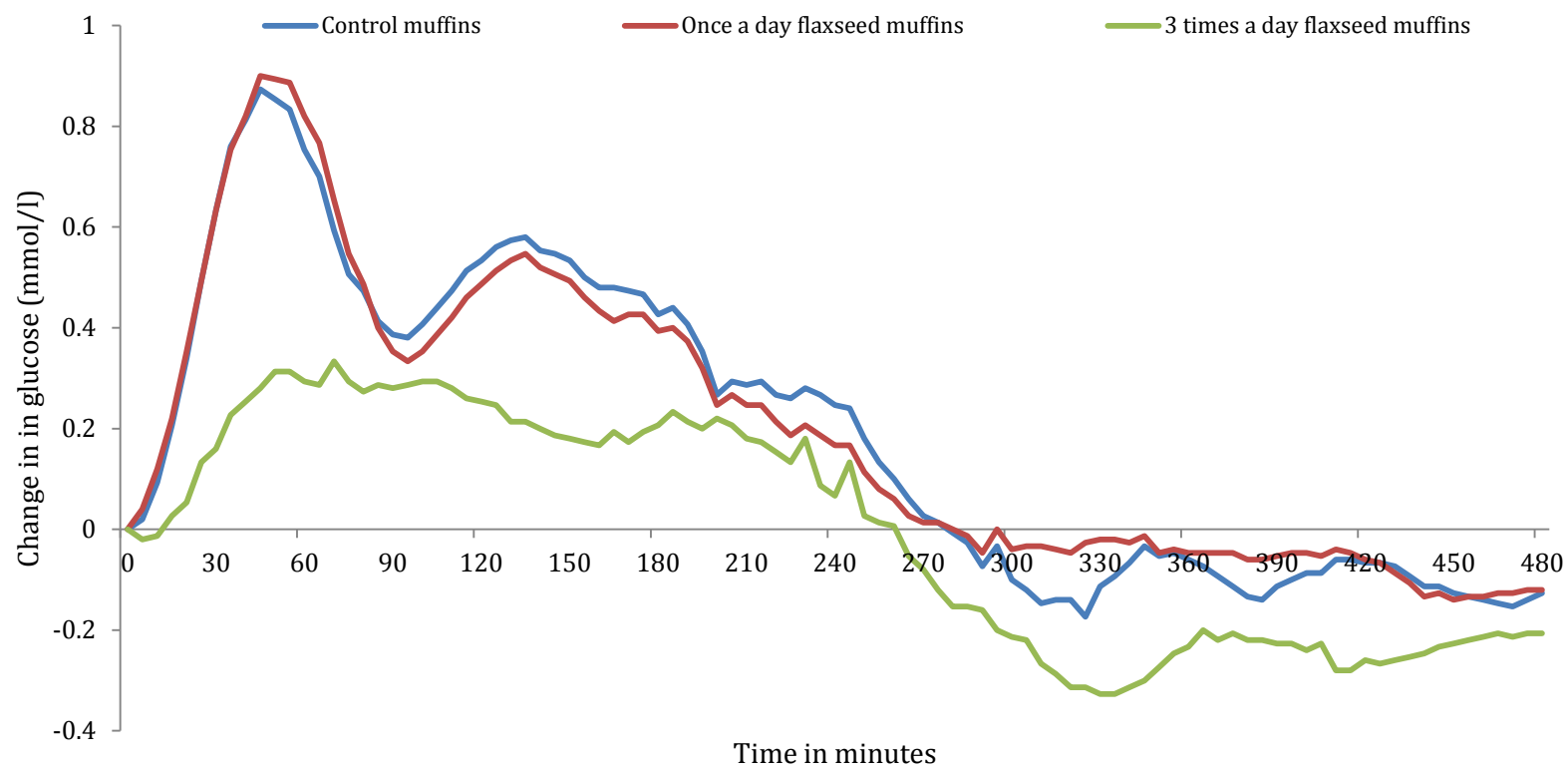


Figure 5.8 Mean change in blood glucose (mmol/l) 8 hours from dinner to the next day for each test day calculated as the mean change in blood glucose from the baseline, which was the time of consuming the dinner (n=15)

5.3.3 Blood glucose iAUC

The blood glucose iAUC results (Table 5.5) show that adding flaxseed muffins to the diet whether in one portion or split over 3 times a day, had the effect of lowering the iAUC for the glucose over 24 h ($p=0.014$) between the control muffins and flaxseed three-times-a-day and during the night ($p=0.005$) between the control muffins and both flaxseed treatment (flaxseed once a day and flaxseed three-times-a-day); however, there was no significant difference between the flaxseed treatments.

Table 5.6. Blood glucose iAUC (mmol.min/l) for each test day (mean $\pm$ SD)

	Control muffins mmol/l	Once a day flaxseed muffins mmol/l	Three times a day flaxseed muffins mmol/l	<i>P</i> values
iAUC (6.00-6.00 h)	1760.1 $\pm$ 1087.7	1214 $\pm$ 1065.4	893.8 $\pm$ 692.8 ^a	0.014
iAUC(6.00-22.00h)	1207.0 $\pm$ 728.2	931.7 $\pm$ 787.6	689.6 $\pm$ 448.4	0.127
iAUC Day AM (6.00-12.00 h)	312.8 $\pm$ 183.9	287.4 $\pm$ 245.0	224.4 $\pm$ 139.8	0.627
iAUC Day PM (12.00-22.00 h)	768.8 $\pm$ 478.3	648.9 $\pm$ 564.2	470.3 $\pm$ 352.8	0.057
iAUC (22.00-6.00 h)	553.1 $\pm$ 382.3	282.3 $\pm$ 292.4 ^a	204.2 $\pm$ 276.5 ^a	0.005

^a= Significant difference ($p<0.05$) compared to control muffins.

Repeated measures ANOVA was performed followed by Bonferroni for multiple comparisons.

5.3.4 Absolute blood glucose level

The results for the absolute blood glucose levels (Table 5.7) according to each treatment (control muffins, once-a-day flaxseed muffin and three-times-a-day flaxseed muffin) over 24 hours, over the day and over the night. There were no significant differences between the treatments for any examined time period , except for the night (22.00-6.00 h) where there was a significant reduction ($p = 0.011$) in blood glucose concentration after consuming flaxseed once(4.8 ± 0.3 mmol/l) compared to the control muffins (5.2 ± 0.1 mmol/l).

Table 5.7. Blood glucose concentration (mmol/l) over 24 hours, day and night for each test day (mean $\pm$ SEM)

	Control muffins mmol/l	Once a day flaxseed muffins mmol/l	Three times a day flaxseed muffins mmol/l	<i>P</i> values
24 hour (6.00-6.00 h)	5.2 ± 0.1	5.1 ± 0.1	5.2 ± 0.1	0.419
Day (6.00-22.00 h)	5.2 ± 0.1	5.3 ± 0.1	5.4 ± 0.1	0.436
Day AM (6.00-12.00 h)	4.9 ± 0.2	5.0 ± 0.2	5.3 ± 0.1	0.175
Day PM (12.00-22.00 h)	5.5 ± 0.1	5.4 ± 0.1	5.4 ± 0.1	0.745
Night (22.00-6.00 h)	5.2 ± 0.1	4.8 ± 0.3^a	5.0 ± 0.1	0.011

^a = Significant difference ($p < 0.05$) compared to control muffins.

Repeated measures ANOVA was performed followed by Bonferroni for multiple comparisons.

The results for the blood glucose mean levels pre and post main meals which were calculated one hour before each meal and one hour after each main meal (Table 5.8) showed were no significant differences between any of the treatments (control muffins and flaxseed muffins, once and three times a day).

Table 5.8. Blood glucose concentration (mmol/l) pre and post main meals for each test day for each test day (mean $\pm$ SD)

	Control muffins mmol/l	Once a day flaxseed muffins mmol/l	Three times a day flaxseed muffins mmol/l	<i>P</i> values
Before breakfast	4.6 $\pm$ 0.8	4.6 $\pm$ 0.7	5.0 $\pm$ 0.6	0.082
After breakfast	5.6 $\pm$ 0.8	5.5 $\pm$ 1.0	5.7 $\pm$ 1.0	0.538
Before lunch	5.3 $\pm$ 0.6	5.3 $\pm$ 0.6	5.3 $\pm$ 0.6	0.665
After lunch	5.7 $\pm$ 0.7	5.5 $\pm$ 0.7	5.6 $\pm$ 0.6	0.613
Before dinner	5.0 $\pm$ 0.5	5.0 $\pm$ 0.4	5.1 $\pm$ 0.4	0.403
After dinner	5.6 $\pm$ 0.7	5.6 $\pm$ 0.7	5.3 $\pm$ 0.7	0.134

(Repeated measures ANOVA was performed followed by Bonferroni for multiple comparisons).

5.3.5 Blood glucose peak values and time to peak

The peak and time to peak pre and post meals (Table 5.9) were consistent with the results of glucose levels pre and post meals (Table 5.8) and showed that there were no significant differences between all treatments. However, the breakfast blood glucose peak value for flaxseed muffins once (6.0 mmol/l) was lower than the other treatments (control and flaxseed muffins 3 times) (6.4 mmol/l for both).

Table 5.9. Blood glucose peak and time to peak for each main meal for each test day (mean $\pm$ SD).

	Control muffins	Once a day flaxseed muffins mmol/l	Three times a day flaxseed muffins mmol/l	<i>P</i> values
Peak breakfast (mmol/l)	6.4 $\pm$ 0.8	6.0 $\pm$ 0.8	6.4 $\pm$ 1.0	0.207
Peak lunch (mmol/l)	6.4 $\pm$ 0.8	6.4 $\pm$ 1.0	6.4 $\pm$ 0.8	0.920
Peak dinner (mmol/l)	6.3 $\pm$ 0.4	6.4 $\pm$ 0.9	6.1 $\pm$ 0.7	0.307
Time to peak breakfast (min)	9.52 $\pm$ 1.5	9.50 $\pm$ 1.3	9.57 $\pm$ 1.6	0.970
Time to peak lunch (min)	15.00 $\pm$ 2.0	14.37 $\pm$ 1.7	14.45 $\pm$ 0.1	0.420
Time to peak dinner (min)	18.27 $\pm$ 6.6	19.50 $\pm$ 4.1	20.00 $\pm$ 4.7	0.169
Repeated measures ANOVA was performed followed by Bonferroni for multiple comparisons.				

5.3.7 Gastrointestinal symptoms

The effect of adding flaxseed to muffins on gastrointestinal symptoms 24 hours post consumption was investigated and the results (Figure 5.9 and 5.10) for the frequency and intensity for all the symptoms were similar for both flaxseed treatments, with significant differences between both flaxseed treatments and the control muffins ($p < 0.001$) for the frequency of abdominal cramping and flatulence.

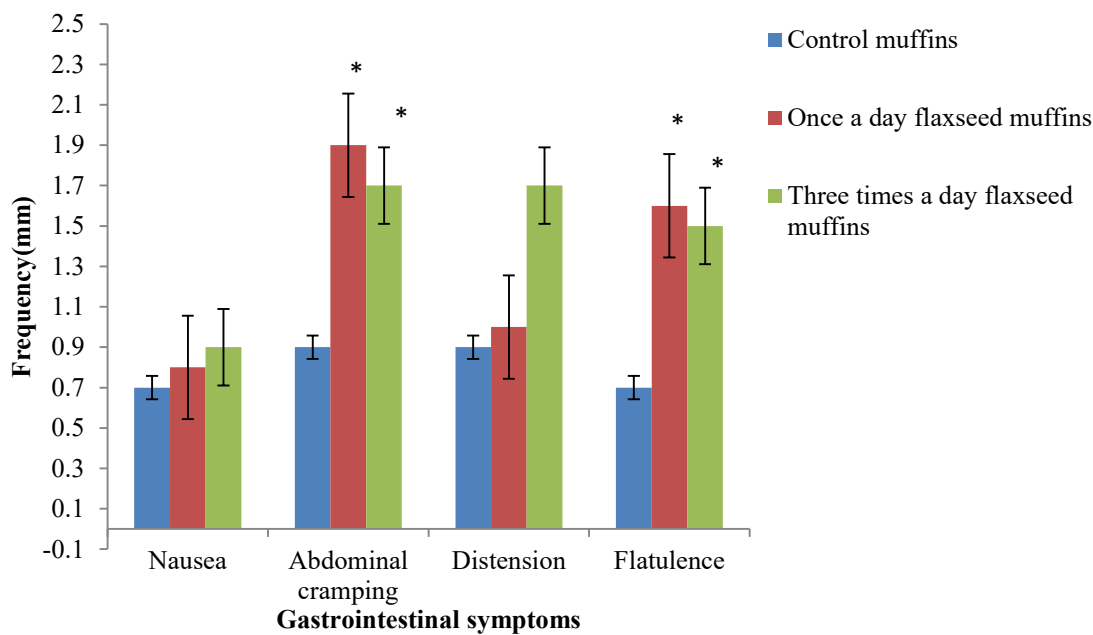


Figure 5.9. Mean frequency of some gastrointestinal symptoms 24 hours post consumption for each test day (n=15).

*= Significant difference ($p < 0.05$) compared to control muffins.

Repeated measures ANOVA was performed followed by Bonferroni for multiple comparisons.

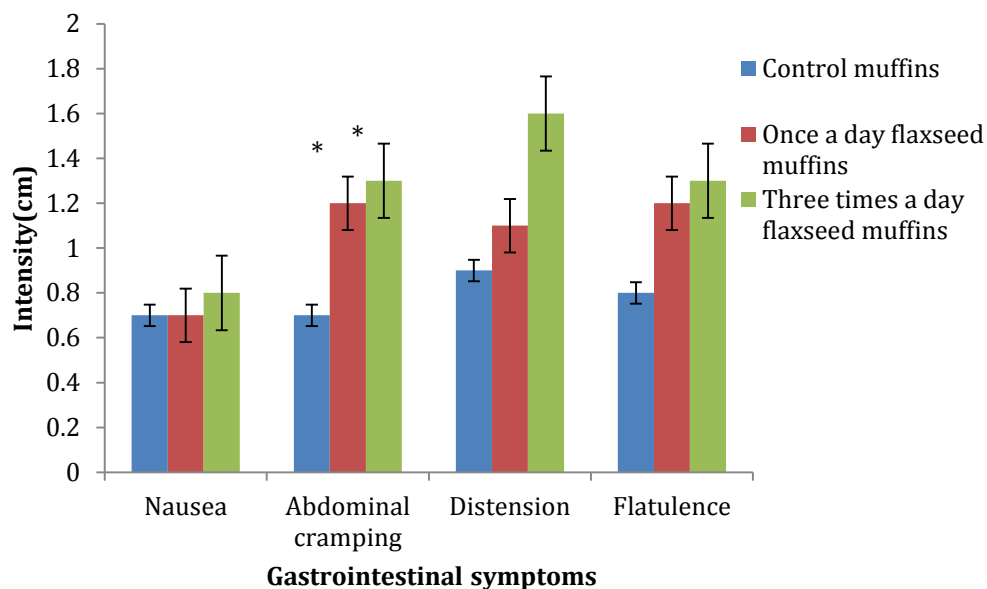


Figure 5.10. Mean intensity of some gastrointestinal symptoms 24 hours post consumption for each test day (n=15).

*= Significant difference ($p < 0.05$) compared to control muffins.

Repeated measures ANOVA was performed followed by Bonferroni for multiple comparisons.

5.4 Discussion

The present study aimed to investigate the effect of adding flaxseed to muffins in one single portion or split into three portions three times a day, on blood glucose profile using CGMS among healthy participants. The results of this study demonstrated that flaxseed muffins were effective at lowering and maintaining blood glucose levels over 24 hours, when consumed once a day or three times a day, compared to the control muffins. These results may be attributed to the total macronutrients and fibre in flaxseed muffins in both treatments; these muffins were higher in total fibre, protein and lower in carbohydrate compared to the control muffins (see Section 5.2.5 Table 5.5) as well as higher in TPC (see Chapter 2 Section 2.3.1). Moreover, the components of flaxseed, which include of lignan and soluble fibre which can had an impacts on insulin sensitivity and blood glucose control (Hallund *et al.*, 2008; Au *et al.*, 2013). The absorption of glucose may be reduced by the fibre content of the flaxseed, thereby moderating the insulin production requirement. Flaxseed ingestion, therefore, may reduce insulin secretion (Hutchins *et al.*, 2013; Morisset *et al.*, 2009). Additionally, omega-3 fatty acids in the form of ALA are contained within flaxseed, which have a positive impact upon glycaemic control (Au *et al.*, 2013; Hallund *et al.*, 2008). Moreover, ALA may have an anti-inflammatory effect which has been linked to enhanced insulin sensitivity in the liver, muscle and adipose tissue, and to modulation of incretin hormones which are linked to the glucose-stimulated insulin secretion (Flachs *et al.*, 2009).

In the results for the blood glucose profile over 2 hours after breakfast (Figure 5.6) it can be seen that there was a delay in the blood glucose values compared to the plasma

glucose results in Chapter 4 (see Section 4.3.2.2, Figure 4.1). These differences may be due to the difference between plasma glucose compared to interstitial fluid. For example, Freckmann *et al.* (2007) performed a study assessing the blood glucose profile for 24 hours among non-diabetic participants by continuous glucose monitoring sensors and venous blood sampling. It was found that the interstitial glucose concentration for the 24 hours was lower than in the venous blood with a mean of 89.3 ± 6.2 mg/dl and 90.9 ± 6.8 mg/dl (16 values per 24 hours).

Glucose measured in interstitial fluid may show a considerable difference from blood glucose measured simultaneously (Kulcu *et al.*, 2003). A delay of between 5 to 10 minutes is apparent in the interstitial fluid glucose response to blood glucose changes. Such variations may indicate physiological changes in utilisation and elimination in blood, cells and interstitial space as well as glucose uptake (Basu *et al.*, 2013). Nevertheless, interstitial fluid glucose is lower, lagging behind blood glucose, because it is necessary to transfer glucose from the blood to the interstitial space. Moreover, as the blood glucose rate increases, the difference is greater, whereas both compartments equilibrate at a steady state in which the difference is minimal. (Kulcu *et al.*, 2003; Basu *et al.*, 2013).

Furthermore, in the blood glucose values 2 hours post breakfast (Figure 5.5), it can be seen that there was a lack of difference at breakfast between the test days (control muffins and flaxseed muffins, once and three times a day) compared to the lunch and dinner. This may be explained by the fact that the baseline on that chart was 6.00 am, regardless of the time of the meals. The participants were instructed to consume breakfast between 7.00 and 9.00am, so some of the participants might be in a fasting state at that time, as every participant may have consumed their meal a different time

within the suggested timeframe. There was a difference between the test days 2 hours post breakfast (Figure 5.6); this was because the baseline was calculated for each participant separately, depending on the time of breakfast, which was the baseline, so this standardised the breakfast time. Therefore the data were calculated based on all participants being in a postprandial state.

To the best of our knowledge there is currently no previous study has investigated the effect of flaxseed on the glucose profile over 24 hours. To discuss these results this effect may be linked to the hypothesis that the flaxseed muffins had a lower GI as concluded in Chapter 3 of this thesis. Moreover, the results from Chapter 4 also indicated that flaxseed muffins were effective in lowering GR.

Reliable evidence implies that the consumption of low GI food reduces large blood glucose fluctuations (Kaur *et al.*, 2016; Brand-Miller *et al.*, 2003). Many studies have looked at the effect of low GI food on the blood glucose profile over 24 h among healthy participants such as the studies by Bynel *et al.* (2005), Henry *et al.* (2006), Henry, Newens, and Lightowler. (2009) and Kaur *et al.* (2016). All of the graphs for the blood glucose profile over 24 hours for the above studies showed lower values for low GI food, especially during the night (22.00-06.00h) and this is consistent with the results of this study for the glucose profile graph over 24 hours as both flaxseed treatments showed lower values than the control, despite the control muffins having a medium (not high) GI of 64.3. It has been deduced from various studies that low GI foods ingested at breakfast reduced glycaemia after a subsequent standardised lunch; a phenomenon known as the “second-meal effect” . In the short and medium terms, the consumption of food which causes a second-meal effect may assist in sustaining

low blood glucose concentrations, consequently reducing demands upon the insulin-mediated blood glucose regulatory systems (Kaur *et al.*, 2015).

Low GI meals produced a lower GR compared to higher GI meals, with a higher glucose excursion after breakfast compared to dinner or lunch, due to insulin sensitivity being higher in the morning (Shih *et al.*, 1992). The results of this study are consistent with the study of Henry *et al.* (2006) both studies found the low GI food can improve the glucose profile over a 24 h period. These changes if applied over the long term could potentially improve glucose control, which could result in a reduced chronic disease risk such T2D and CVD (Henry *et al.*, 2006).

This implies that first meal of the day (breakfast) may be the largest contributor regarding daily GR and also that there may be an improvement in glucose tolerance for subsequent meals such as lunch and dinner (Wolever *et al.*, 1988; Nilsson *et al.*, 2008; Fletcher *et al.*, 2018). Furthermore, this may cause a lower glucose excursion following a breakfast, since there is a greater insulin sensitivity in the morning than there is in the afternoon (Shih *et al.*, 1992). A possible mechanism is that breakfast may serve as primer to the insulin-sensitive tissues either by: (1) adjusting b-cell responses with changed incretin signalling or (2) an insulin sensitivity change following breakfast (Park *et al.*, 2015). Although the results of the current study did not show differences after each of the individual meals, this effect is applicable to the current study as the results of iAUC over 24 hours was significantly lower when flaxseed muffins were consumed three times a day compared to the control.

The results for blood glucose iAUC during the night indicated that both flaxseed treatments produced lower readings than the control muffins and this agrees with the

results of Brynes *et al.* (2005), who found that low GI food decreased overnight blood glucose level by 16 %. In the current study, this result could be due to the effect of flaxseed on fasting blood glucose, which can be linked to the amount of dietary fibre in the flaxseed muffins. The flaxseed muffins contained 4.9 g /100 g of fibre with low GI (42.2) and this could make them more effective at lowering blood glucose.

These results could be important, as other studies have shown that changes in GI were generally improved postprandial glucose concentrations, compared to fasting concentrations. Studies have shown that low-GI diets in healthy adults can significantly lower fasting plasma glucose levels, which suggests that by switching to a low-GI diet an improvement in hepatic insulin sensitivity that would effectively decrease the hepatic glucose output (Brynes *et al.*, 2005). Consumption of a high GI food can lead to hypoglycaemia during the night, whereas by meals based on low GI foods mean the blood glucose level can be better maintained (Liu *et al.*, 2012).

The impact of the reduction of blood glucose is a basic element of a low-GI diet and the mechanisms underlying the metabolic impacts are multifactorial; they could also involve: glucose absorption, a reduced gastric emptying rate, the food matrix, a slower small-intestinal as well as a decreased insulin response (Wolever, 1990a). As mentioned above, a low GI reduced the total 24-hour glucose, irrespective of whether the high-energy meal was consumed in the morning or in the evening (Brynes *et al.*, 2005).

For the absolute glucose levels over the full 24 hours, there were no significant differences between any of the treatments in this study, which agrees with the results of the study by Aston *et al.* (2010) on the effect of low and high GI diets among 12

overweight women. The results show a similar trend even though the current study was among both males and females and did not use high GI muffins, as the control muffins were medium GI. The results for the glucose values pre- and post- meals in this study showed no significant difference between treatments, which does not agree with Kaur *et al.* (2016), who found that these values for the low GI meals were significantly different when compared to the high GI meals. The change from the baseline at breakfast was 0.07 mmol/l in the current study for the flaxseed muffins 3 times compared to 0.46 mmol/l for Kaur *et al.* (2016) which used 12 participants, for lunch Kaur *et al.* (2016) found was 0.76 mmol/l compared to 0.03 mmol/l in the current study. The difference in values may be due to the fact that Kaur *et al.* (2016) calculated the difference from the baseline values, while in the current study the results were calculated from 2 hours post each meal.

The current study also investigated gastrointestinal symptoms compared with the control muffins, which caused few such side effects and found that flaxseed muffins of both treatment regimens caused some side effects on the gastrointestinal system. This is consistent with the study in Chapter 4 in this thesis on the effect of whole and ground flaxseed muffins on gastrointestinal symptoms 24 hours post consumption, as well as with the results of Kristensen *et al.* (2017), who found that 5 g flaxseed dietary fibre supplementation for 12 weeks (with or without the addition of calcium) resulted in some gastrointestinal symptoms, especially abdominal pain, flatus and diarrhoea. The effect of flaxseed muffins in the current study and flaxseed fibre in Kristensen *et al.* (2017) may be due to the amount of dietary fibre in the flaxseed. These results suggest flaxseed muffins can cause some gastrointestinal side effects, although they were not severe according to the results of the questionnaire, as the highest value was only 1.9 on a scale of 1-10.

5.5 Summary

The results of this study indicated that adding flaxseed of a day produced a lower glucose profile over 24 hours in a free-living context compared to the control muffins.

All the blood glucose results from this study indicate that flaxseed in both flaxseed treatments was effective in lowering and stabilising blood glucose levels during the day and night, compared to the control muffins. These results were in line with the results of the glycaemic index study (Chapter 3) and glycaemic response study (Chapter 4), which showed that flaxseed muffins had a lower GI and were effective in lowering the GR, compared to the control.

The results of both flaxseed treatments (flaxseed once or three-times-a-day) found that there was no significant difference in any of the results between the two flaxseed treatments. Furthermore, both treatments were effective in enhancing the blood glucose profile over the 24 hours and during night. That is means that the amount of 30 g of flaxseed per day was effective regardless of whether this amount was split across meals or taken or having them once a day.

These studies provide important data about adding flaxseed to the diet for healthy individuals, which can enhance the glycaemic profile during the day.

Chapter 6 : General discussion and conclusion

6.1 General discussion and findings

Flaxseed has recently become the focus of many studies due to its health benefits as a functional food, especially as it is considered to be one of the highest plant sources of omega-3 fatty acids and lignan. Flaxseed also contains high amounts of fibre, proteins, amino acids and vitamin E, and can be a useful supplement to the human diet (Goyal *et al.*, 2014; Chishty and Monika, 2016). Flaxseed also has antioxidant properties which can lower plasma glucose and HbA1c concentrations (Cunnane *et al.*, 1993); thus, there may be a role for flaxseed in lowering the risk factors of diabetes, especially in patients with obesity and dyslipidaemia (Tarpila *et al.*, 2005; Morisset *et al.*, 2009).

Until now, most previous research studies have focused on diabetic or hyperlipidaemic individuals, however there may be a rationale to encourage regular consumption of flaxseed among healthy individuals as a part of a healthy diet. It has been suggested that consumption of flaxseed in different forms, whether as an oil or flaxseed-enriched products, would be suitable for use as a wide-scale public health intervention as its inclusion in the diet would greatly increase omega-3 fatty acids, fibre and lignan intake, which could help contribute to the prevention of the onset of chronic diseases such as T2D and CVD (Kontogianni *et al.*, 2013; Bernacchia *et al.*, 2014).

There was therefore a need for further studies to investigate the effect of incorporating flaxseed into products (e.g. bakery products) on the glycaemic profile of healthy

individuals, as there is a surprisingly limited amount of literature considering the effect of flaxseed on GR and IR.

Four studies were conducted to complete this thesis; these consisted of both *in vitro* (Chapter 2) and *in vivo* studies (Chapters 3, 4 and 5). The results from the *in vitro* study (Chapter 2) show that ground flaxseed produced significantly higher levels of both TPC and antioxidant capacity, and both flaxseed forms reduced the RDS in the muffins, which could lead to lower blood glucose levels. All the data from the *in vitro* study supported the decision to conduct *in vivo* studies on the effect on the blood glucose levels in human participants. The results from Chapter 3 were in line with the results of Chapter 2, showing that muffins made with ground flaxseed had a lower GI compared to the other muffins. The results from Chapter 4 support the results from Chapter 3 on the effect of flaxseed on the blood glucose, as well as adding data on the effect of flaxseed on insulin and satiety, providing a better understanding of the effect on the blood glucose profile.

As hypothesised, flaxseed had an effect on the glucose and insulin levels (Chapter 4), because it had higher amounts of TPC and antioxidants as well as having smaller amounts of RDS (results from Chapter 2) and having lower GI values (Chapter 3) compared to control muffins. All results from Chapters 3 and 4 determined that flaxseed was effective; the remaining question was whether flaxseed muffins were more effective, when given once or more than once during the day, on the 24-hour blood glucose profile in healthy participants, using a continuous glucose monitoring system. The results from Chapter 5 indicated that both flaxseed treatments were effective in enhancing the blood glucose profile over the 24 hours, compared to control muffins.

To the best of our knowledge, the *in vitro* study in Chapter 2 was novel as there are no earlier data comparing TPC and total antioxidant capacity, *in vitro* starch digestion and sugars released in different forms of full fat flaxseed (whole and ground) incorporated into bakery products such as muffins. Most previous studies have used defatted flaxseed (e.g. Silva *et al.*, 2012; Kaur and Kaure, 2018). During the de-fatting process, the antioxidant activity of flaxseeds can be lowered, which suggests that using full- fat whole seeds may provide a higher amount of antioxidant capacity (Brodowska *et al.*, 2014). Studies have shown that flaxseeds that have not undergone the de-fatting process had higher levels of free phenolic acids, which comprised over (50%) of the total phenolic compound content (58%), when compared to the insoluble-bound phenolic compounds (7 - 21%) (Alu'datt *et al.*, 2013). This is important as soluble free phenolic acids have a higher antioxidant capacity compared to insoluble-bound phenolic compounds.

The *in vitro* study in Chapter 2 is important as it provides data on the TPC and antioxidant capacity of full- fat flaxseed muffins. Moreover, different epidemiological studies have shown that consuming foods with high polyphenol content daily can be beneficial and protect against diseases, including diabetes, cancer and CVD (Graf, Milbury and Blumberg *et al.*, 2005). The results for the sugar released during *in vitro* ileal digestion of flaxseed muffins showed that the sugar release in both flaxseed muffins was significantly lower ($p < 0.05$) than the control muffin. These results support the hypothesis that the addition of flaxseed will increase antioxidant activity and TPC, which could reduce RDS levels, and this can in turn cause a reduction in blood glucose levels.

The *in vitro* study formed the basis for conducting the *in vivo* studies. To assess the impact of flaxseed on the GR, three *in vivo* studies were carried out to determine the GI (Chapter 3), the effect of flaxseed on GR/IR (Chapter 4) and the effect of a split portion of flaxseed on the 24 h blood glucose profile (Chapter 5). Overall, these studies concluded that flaxseed muffins had a lower GI and were effective in lowering GR and IR compared to the control muffin.

Bakery products such as bread and muffins are generally classed as medium-to-high GI foods due to the fact they are cereal products that contain high levels of white flour and sugar; therefore being able to reduce the GI of these products which are frequently consumed would be beneficial on health (Ferrer-Mairal *et al.*, 2011). It has been shown that fibre can reduce GI values and this is especially true for soluble fibres such as mucilage, pectin and gums (Wolever *et al.*, 1990a; Vinoy *et al.*, 2017). A good example of a seed that contains a high fibre content is flaxseed, as it contains 28% dietary fibre with the main source of soluble fibre being mucilage gum. Therefore, it was important to assess the GI of the flaxseed muffins to determine whether they had a low GI value which could contribute to lower blood glucose levels post-consumption (Chapter 3).

This study was important as it provided data on the GI value of muffins containing flaxseed in different forms (ground and whole) and showed that both forms had a lower GI compared to the control muffins. To the best of our knowledge, there is no previous data available in the literature comparing different flaxseed forms incorporated into bakery products, especially like in this study where 48 % of the wheat flour was replaced with flaxseed. However other available data on flaxseed added to food products can be found in the international tables for GI values by

Atkinson *et al.* (2008) with several examples demonstrating that flaxseed reduce the GI of foods such as bread and breakfast cereals.

The results from the GI study (Chapter 3) supported the hypothesis that flaxseed has an effect on glucose and insulin levels which was the starting point for the GR/IR study (Chapter 4). The main result from this study on glucose and insulin levels (Chapter 4) confirmed that flaxseed muffins did indeed produce a lower GR and IR compared to a control muffin. These effects can be explained by the fact that flaxseed contains both soluble and insoluble fibre as well as lignan which has been shown to play a key role in lowering postprandial blood glucose levels (Cunnane *et al.*, 1993; Qian *et al.*, 2012). These results also agree with previous studies into changes in insulin concentration, such as those by Kristensen *et al.*, (2013), that showed in healthy men the dietary fibre in flaxseed effectively lowered postprandial insulin levels.

Within the literature, to the best of our knowledge, there are no previous studies that compare the effects of using whole and ground flaxseed in bakery products (for example in muffins) on GR and IR. As both forms of flaxseed (whole and ground) had been shown to be effective in lowering GR and IR, the remaining question was whether flaxseed was more effective when the portion was given all at once or splitting it more than once during the day. To investigate this question, there was a need to monitor blood glucose during the 24 hours post consumption, with collection of the full glucose profile, which was the aim of the final study (Chapter 5). In this study, ground flaxseed muffins were used as the *in vitro* study (Chapter 2) showed that they were higher in TPC and antioxidants. Moreover, the results of the GI study (Chapter 3) showed that these muffins had a lower GI compared to the whole flaxseed

muffins. Although the results for GR/IR (Chapter 4) were not significant, the sensory evaluation results showed ground flaxseed muffins were preferred by the participants.

As with the other studies, to the best of our knowledge, there are no previous studies in the literature that have investigated the effect of splitting the portion of flaxseed on blood glucose, or whether the flaxseed is more effective administered in a single or split-portion. Within the study, the flaxseed portion was split into three to coincide with the three meals consumed during the day, which meant the effect of the flaxseed was spread over time and this could help with lowering the blood glucose level after consumption of each meal. This was the first study specifically designed to study the effect of flaxseed on the blood glucose profile among healthy participants. This study was important as it provided data on the blood glucose profile during a full day under normal living conditions. All results of the three *in vivo* studies (Table 6.1) regarding the glucose iAUC showed that flaxseed was effective in lowering the GR, as well as stabilising blood glucose levels during the day, as all the results were lower than those for the control muffins.

Table 6.1 Summary for all three *in vivo* studies.

	Ch. 3	Ch. 4	Ch.5
	GI study	GR study	Split potion study
Potion of flaxseed (g)	21	30	30
Participants	10	32	15
Blood glucose iAUC 120 min (mmol.min/l)			
Control muffins	87.5	126.4	72.2
Whole flaxseed muffins	78.9	87.4	
Ground flaxseed muffins	63	90	56.1
(Once a day 30g on breakfast)			
Ground flaxseed muffins			68.3
(Three time a day 10g on breakfast)			

From all results it is clear flaxseed was effective with regard to the lowering of GR probably due to components such as polyphenols, fibre, fat and protein which have a role in lowering blood glucose. Polyphenols can inhibit the starch breakdown to glucose by affecting the digestive enzymes such as α -amylase and α -glucosidase (McDougall and Stewart, 2005). This may result in a reduction of the sugar released from the starch in the food and this would reduce the RDS resulted in lower GI values.

The role of fibre can be seen in reducing the sugar released from the starch in the food and this would reduce the RDS and lead to lower GI. Dietary fibre that can be dissolved in water alters the speed of starch digestion and degradation by changing the consistency, composition and thickness of food (Brennan, 2005). This effect is associated with the management of postprandial glucose levels.

For instance, the rheological characteristics of viscous fibre means that it coagulates with the food and the products of human digestion, lessening concentrations of postprandial plasma glucose relative to viscosity levels in both diabetic and healthy people (Kim, Wong and Vuksan 1996). Viscous fibre enacts such an effect by slowing the speed at which glucose is digested and absorbed by the blood (Rayner *et al.*, 2001). Consequently, fibre in a more viscous mixture substantially lowers blood glucose levels (Jenkins *et al.*, 2008). During digestion, dietary fibre is processed using a different pathway to other types of CHO. In the small intestine dietary fibres are not fully digested, which means blood glucose levels are lower after their consumption than after ingestion of other types of CHO (Tappy *et al.*, 1996). The four pathways most commonly suggested are: delayed gastric emptying, modulated release of the gut hormones that regulate digestion and absorption, reduced digestive enzyme activity in the small intestine and delayed diffusion of hydrolysed starch fragments or glucose in the small intestine (Dikeman and Fahey, 2006; Grundy *et al.*, 2016).

Protein in the food matrix may reduce the starch digestion rate; the interaction of proteins and starches significantly affected their digestibility. Starch can also form complexes with lipids, which can cause significant behavioural changes in the starch, including delayed retrogradation, increases in gelatinization temperature, decreased solubility and resistance to being digested by enzymes (Crowe, Seligman and Copeland, 2000 ; Singh Dartois, and Kaur 2010).

It is believed that flaxseed has a hypoglycaemic impact because of all the above compounds, and in the case of Type 2 diabetic patients, this has the ability to enhance the lipids profile and blood glucose (Pan *et al.*, 2009). Consequently, when flaxseed is included in Type 2 diabetes patient's diets, it assists in regulating dyslipidemia and

hyperglycaemia, ultimately leading to a reduction in secondary diabetes complications, enhancing life quality. Such hypoglycaemic action should avoid or reduce the possibility of diabetic persons developing secondary diabetes complications. Flaxseed supplementation has previously shown to be of assistance in regulating hyperglycaemia and enhancing lipid profiles (Mani *et al.*, 2011).

6.2 Study applications and implications

The findings from these studies have important implications that can help contribute to further research. Firstly, the beneficial effect of flaxseed on the GR, IR and 24-hour blood glucose profile in healthy adults (Chapters 4 and 5) may come from direct actions relating to their high polyphenol or fibre content (Chapter 2). Furthermore, including flaxseed could enhance the quality of a healthy individual's diet by adding to the soluble fibre content, thus improving glycaemic control and increasing the overall fibre intake in healthy individuals. Adding flaxseed as part of a healthy diet alongside physical activity can be a prevention tool against developing Type 2 diabetes and cardiovascular disease.

Despite the health benefits of flaxseed, awareness is lacking among the general public of the importance of the consumption of flaxseed and how it can be added to the diet directly or incorporated within food products (Gutte *et al.*, 2015). Health providers and dieticians should encourage overweight and obese people to use flaxseed, in either whole seed or ground form, as a dietary supplement because of its health benefits preventing Type 2 diabetes and cardiovascular disease. Flaxseed can be incorporated into the everyday diet by adding it to many different products, such as breakfast cereals and baked goods, either as whole or ground flaxseed; it can be added

into salad dressings or added into yogurts. This could encourage manufacturers and bakeries to develop new foods, branded as healthy and functional, using flaxseeds in different forms: whole, ground and oil.

6.3 Study limitations

A limitation of the first study (Chapter 2) was that it used the determination of the TPC in the flaxseed muffins rather than a specific type of polyphenols, such as lignan which is the main polyphenol in the flaxseed, due to financial constraints. Knowing the levels of the specific compounds gives a more precise picture of the differences in polyphenol content, which is important as we aim to improve the body of evidence around the health effects of individual compounds; however, we do not yet fully understand how each individual compound interacts within the food matrix and other polyphenols due to the complexity of our diets (Opara and Chohan, 2014).

One of the limitations of third study (Chapter 4) was that it was a short-term study for the measurement of GR, IR and satiety, only measuring these values for two hours post consumption. A short-term study does not represent the full effects of flaxseed on metabolic health, which includes measurement of lipid profiles, as well as insulin resistance index (HOMA-IR), HbA1c, blood pressure and weight changes. All of these measurements need a longer-term study, as well as needing the compliance of the participants. The other limitation was that flaxseed was incorporated into bakery products only; therefore, there is a need to compare the effect of having flaxseed in other forms, such as flaxseed oil, or flaxseed extract lignans capsules to help fully elucidate the best way to deliver the health benefits of flaxseed.

One of the limitations in the fourth study (Chapter 5) was that it lasted for just one day, due to financial constraints. The study was reliant on the participants following all the instructions; it would have been easy for them to misreport the time that they had the meals or the time that they did the capillary finger-prick. They might also have forgotten to do a capillary finger-prick, and as it is crucial for the calibration of the CGMS to have glucose data, this could result in missing data. However, in this study the compliance of participants was good, all the data for the capillary finger-pricks were completed and there were no missing data in terms of blood glucose. This was therefore not a limiting factor.

6.4 Further studies

The study provided important data on TPC and antioxidants in flaxseed muffins as well as the *in vitro* starch digestion and its effect on the glycaemic profile. However, there is a need to investigate the lignan content of flaxseed, which is one of the bioactive compounds in the flaxseed. More studies are needed to assess the TPC and antioxidants in other flaxseed forms, such as flaxseed isolated protein, mucilage and derived lignan, as ingredients to be added to products such as bakery products. Regarding the *in vitro* starch digestion, there is a need to conduct more studies to determine the RS in the flaxseed muffins; moreover, there may be a need to conduct *in vitro* digestion for other flaxseed compounds such as protein and fat.

This study provides GI values for the flaxseed muffins; more research is needed to provide data on GI values for flaxseed incorporated into bakery products using different forms of flaxseed such as flaxseed flour and flaxseed mucilage, with different amounts added.

The participants in the different studies were all healthy; this study could therefore be extended by carrying out long-term research using different groups of participants, including those who have T2D, pre-diabetes and insulin intolerance, to see if the effects are similar. Moreover, more studies are needed on the effect of flaxseed on metabolic health, which includes measurement of lipid profiles, as well as insulin resistance index (HOMA-IR), HbA1C, blood pressure and weight changes.

This study has provided some insight into the effect of flaxseed on satiety, but more research is needed, such as using actual food intake measures (for example, an *ad libitum* meal), using 100 mm scales and more parameters for VAS, rather than the category scales used in this study. More studies are needed also to assess the effectiveness of flaxseed on satiety hormones such as leptin and ghrelin, which might provide a full understanding of the effect on satiety.

As flaxseed has been suggested as an aid to weight loss, further research could include studies using overweight and obese individuals to investigate if adding flaxseed to the diet could facilitate weight loss. Further studies could also investigate the effects of flaxseed on weight change, glucose, lipid profiles and insulin, to investigate the effect on metabolic syndrome over the long term.

This study indicated that flaxseed was effective in maintaining the blood glucose during 24 hours among healthy individuals. More studies need to be done using CGM sensors among T2D and pre-diabetes patients for more than one day, with more biomarkers assessed as well as the blood glucose profile, such as the lipids profile, weight change and insulin, as well as satiety.

6.5 Conclusions

Previous studies have demonstrated that flaxseed may be effective at lowering the GR in healthy, prediabetic, diabetic or hypercholesterolaemic participants. Moreover, flaxseed may be effective in different forms in bakery products, such as muffins or bread and in powder form or as flaxseed lignan capsules.

The results of this PhD study have provided new information about the effects of flaxseed in healthy individuals with regard to the effects on the blood glucose profile over 24 hours, glucose-regulation, satiety and insulin levels. These results add to the current body of evidence in the field of functional foods and support the use of such functional food ingredients in a healthy diet, specifically as a way to improve the regulation of glucose in adults.

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Appendices

Appendix 1 a: Methods used to analyses the composition of muffins



Z/001: The determination of nitrogen and crude protein using Leco instrumentation (UD001)

The sample is analysed by a Leco instrumentation following Dumas procedure: The sample is combusted in an oxygen atmosphere, the gaseous product is cleaned and nitrogen compounds converted to nitrogen which is measured by a thermal conductivity cell. The crude protein is calculated by multiplying by the appropriate conversion factor.

Accredited to BS/EN ISO/IEC 17025:2005. UKAS 0680

LOQ 0.1 g/100g

The method of uncertainty with a coverage factor of 2 (ie 95% confidence level)

Relative Uncertainty +/- 2.46%

Z/002: The determination of nitrogen and crude protein – Kjeldahl procedure (UD001)

The sample is digested with a mixture of concentrated sulphuric acid and potassium sulphate using copper (II) sulphate as a catalyst, converting organic nitrogen present to ammonium sulphate. Excess sodium hydroxide is automatically added to the cooled digest to liberate ammonia from the ammonium sulphate.

The ammonia is auto distilled into an excess of auto-dispensed indicator boric acid solution, then auto-titrated with standard sulphuric acid solution. The nitrogen content of the sample is calculated from the amount of ammonia produced. Oxidised forms of nitrogen are normally lost in normal Kjeldahl digestions but this may be prevented by a chromium powder reduction pre-treatment.

Sample with a high fat content are analysed in a kjeldahl flask.

Accredited to BS/EN ISO/IEC 17025:2005. UKAS 0680

Ref: BS EN ISO 5983-2:2009

LOQ 0.1 g/100g

The method of uncertainty with a coverage factor of 2 (ie 95% confidence level)

Relative Uncertainty +/- 3.34%

H/085: The determination of total dietary fibre by the AOAC method ((H/085)

The sample is weighed and de-fatted if necessary. It is then gelatinised and treated with α -amylase and further digested enzymatically with protease and amyloglucosidase to remove the starch and protein. The dietary fibre is precipitated with IMS, filtered, washed, dried and weighed. Total dietary fibre is then determined gravimetrically and corrected for protein and ash.

Accredited to BS/EN ISO/IEC 17025:2005. UKAS 0680

Ref: **AOAC Edition 18. Official Method 991.43.**

LOQ 0.5 g/100g

The method of uncertainty with a coverage factor of 2 (ie 95% confidence level)

Relative Uncertainty +/- 19.33 At 10% level
+/- 30 At 1% level

H/050: The determination of Starch (UD012)

Determination of starch and high molecular weight degradation products of starch in feeding stuffs and milk powders. It is not applicable to feeding stuffs containing beet chips, beet pulp, dried beet tops or leaves, potato pulp, dried yeasts, products rich in inulin (e.g. dried or powdered Jerusalem artichokes) and products containing greaves.

The method consists of two separate determinations. The sample is treated with warm diluted hydrochloric acid, clarified and filtered; the optical rotation of the resulting solution is determined. In the second determination, the sample is extracted with 40% ethanol and filtered. The filtrate is acidified with hydrochloric acid, clarified and filtered again; the optical rotation of the resulting solution is determined at 20°C.

Accredited to BS/EN ISO/IEC 17025:2005. UKAS 0680

Ref: Commission Regulations (EC) No. 152/2009 of 27 January 2009, Section L .COMMISSION DIRECTIVE 1999/79/EC of 27 July 1999 (OJ L209 p23)

LOQ 2 g/100g

The method of uncertainty with a coverage factor of 2 (ie 95% confidence level)

Relative Uncertainty +/- 3.78%

CHROM/215: The determination of fatty acids by GC (UD751)

The lipid fractions of the sample are solvent extracted. The isolated fat is transesterified with methanolic sodium methoxide to form fatty acid methyl esters (FAMES). The FAME profile is determined using capillary gas chromatography (GC). Quantification and identification of individual FAMES in the test material is achieved with reference to calibration standards.

Accredited to BS/EN ISO/IEC 17025:2005. UKAS 0680

LOQ 0.01 mg/100g

Ref: **AOAC Official Method 963.22 'Methyl Esters of Fatty Acids in Oil and Fats'**

The method of uncertainty with a coverage factor of 2 (ie 95% confidence level)

Fatty Acid	Relative Uncertainty ±
FAMES-saturates	0.55% At 62% level
FAMES-monunsaturates	0.91% At 25% level
FAMES-polyunsaturates	6.31% At 5.4% level
FAMES-trans fatty acids	1.59% At 2.7% level

Q/006: Calculations

The following are calculated from the moisture, fat, nitrogen, ash and fibre results;

Group II Available Carbohydrate (UD017) g/100g =
 $100 - (\% \text{Moisture} + \% \text{Fat} + \% \text{Protein} + \% \text{Ash} + \% \text{Dietary Fibre})$

The method of uncertainty with a coverage factor of 2 (ie 95% confidence level), calculated from the uncertainties for moisture, fat, ash and protein on mixed matrices is 39.7 g/100g +/- 1.3 g/100g.

Group II Energy (Human) (UD771) kcal/100g =
 $(4 \times \% \text{Protein}) + (9 \times \% \text{Fat}) + (4 \times \% \text{Available Carbohydrate}) + (2 \times \% \text{Dietary Fibre})$

LOQ 10 kcal/100g

The method of uncertainty with a coverage factor of 2 (ie 95% confidence level), calculated from the uncertainties for available carbohydrates, fat and protein on mixed matrices is 394 kcal/100g +/- 7.4 kcal/100g.

Group II Energy (Human) (UD771) KJ/100g =
 $(17 \times \% \text{Protein}) + (37 \times \% \text{Fat}) + (17 \times \% \text{Available Carbohydrate}) + (8 \times \% \text{Dietary Fibre})$

LOQ 40 KJ/100g

The method of uncertainty with a coverage factor of 2 (ie 95% confidence level), calculated from the uncertainties for available carbohydrates, fat and protein on mixed matrices is 1661 KJ/100g +/- 31.7 KJ/100g.

H/034: The determination of oil in feeding stuffs (UD004)

A homogenised portion of the sample is subjected to solvent extraction with petroleum spirit. The residue is hydrolysed with hydrochloric acid and the oil extracted with petroleum spirit. The oil content is determined gravimetrically.

Accredited to BS/EN ISO/IEC 17025:2005. UKAS 0680

Ref: SI 1999 No 1663



LOQ 0.5 g/100g

The method of uncertainty with a coverage factor of 2 (i.e. 95% confidence level)

Relative Uncertainty +/- 11.71%

H/006: The Determination of Crude Fibre in feedingstuffs (UD009)

A homogenised portion of the sample is defatted and treated successively with boiling solutions of sulphuric acid and potassium hydroxide of specified concentrations. The residue is separated by filtration on a sintered - glass filter, washed, dried, weighed, and ashed. The fibre is determined gravimetrically.

The loss of weight resulting from ashing corresponds to the fibre present in the test sample.

Accredited to Commission Regulation (EC) No 152/2009 27 January 2009

LOQ 0.1 g/100g

The method of uncertainty with a coverage factor of 2 (i.e. 95% confidence level)

Relative Uncertainty +/- 7.36%

Appendix 1 b: Composition of the muffins based on the recipe

Control muffins								
Food Name	Quantity	Energy (Kcal)	Carbohydrate (g)	Protein (g)	Fat (g)	Starch (g)	Fibre (g)	Sugars (g)
Baking powder	0.62	0.98	0.21	0.03	0	0.21	0	0
Bananas flesh only	30	25.7	6	0.36	0.03	0.6	0.42	5.4
Bicarbonate of soda	0.31	0	0	0	0	0	0	0
Butter unsalted	9.4	70	0.06	0.06	7.7	0	0	0.05
Cinnamon ground	0.31	0.41	0.08	0.01	0	0.04	0.16	0.04
Eggs	13.25	17.4	0	1.7	1.2	0	0.21	0
Flour wheat white self raising	31.3	106	22.7	2.8	0.47	22.5	1.3	0.18
Milk semi-skimmed	15.6	7.4	0.7	0.55	0.27	0	0	0.7
Nutmeg ground	0.31	1.4	0.08	0.02	0.11	trace	0.06	0.01
Sugar castor	14.4	60	15.1	0	0	0	0	15.1
Vanilla essence no alcohol	0.63	0	0	0	0	0	0	0
Total	116.13	290	45	5.5	9.8	23.3	2.1	21.5
Whole flaxseed muffins								
Baking powder	0.62	0.98	0.21	0.03	0	0.21	0	0
Bananas flesh only	30	25.7	6	0.36	0.03	0.6	0.42	5.4
Bicarbonate of soda	0.31	0	0	0	0	0	0	0
Butter unsalted	9.37	70	0.06	0.07	7.7	0	0	0.05
Cinnamon ground	0.31	0.41	0.08	0.01	0	0.04	0.16	0.04
Eggs chicken whole raw	13.3	17.4	0	1.7	1.2	0	0.21	0
Whole flaxseed	15	80.1	0.24	2.7	6.3	0.23	4.1	0.23
Flour wheat white self raising	16.25	55	11.8	1.4	0.24	11.7	0.65	0.09
Milk semi-skimmed	15.62	7.4	0.7	0.55	0.27	0	0	0.7
Nutmeg ground	0.31	1.4	0.08	0.02	0.11	trace	0.06	0.01
Sugar castor	14.37	60	15.1	0	0	0	0	15.1
Vanilla essence	0.62	0	0	0	0	0	0	0
Total	116.13	318.4	34.3	6.9	15.9	12.8	5.6	21.6
Ground flaxseed muffins								
Baking powder	0.62	0.98	0.21	0.03	0	0.21	0	0
Bananas flesh only	30	25.7	6	0.36	0.03	0.6	0.42	5.4
Bicarbonate of soda	0.31	0	0	0	0	0	0	0
Butter unsalted	9.37	70	0.06	0.06	7.7	0	0	0.05
Cinnamon ground	0.31	0.41	0.08	0.01	0	0.04	0.16	0.04
Eggs	13.3	17.4	0	1.7	1.2	0	0.21	0
Ground flaxseed	15	87	0.71	3	6	2.26	5	0.2
Flour wheat white self raising	16.3	55	11.8	1.5	0.24	11.7	0.65	0.09
Milk semi-skimmed	15.62	7.4	0.7	0.55	0.27	0	0	0.7
Nutmeg ground	0.31	1.4	0.08	0.02	0.11	trace	0.06	0.01
Sugar castor	14.37	60	15.1	0	0	0	0	15.1
Vanilla essence	0.62	0	0	0	0	0	0	0
Total	116.13	325.3	34.7	7.1	15.6	15.4	6.5	21.6

Appendix 2 a: Participant information sheet

Information Sheet

Glycaemic index (GI) testing of flaxseed muffins



Invitation

You are being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully.

What is the purpose of the study?

Flaxseed is an oilseed which is of interest from both nutritional and medical aspects, due to its special chemical composition and bioactive compounds, which provide health benefits and prevent disease such as cardiovascular disease and type 2 diabetes. Flaxseed can be consumed in many different forms as whole flaxseed, ground meal or extracted oil, making it an appropriate functional ingredient for inclusion in many products, such as baked cereal products, bread, muffins and spaghetti.

This study aims to determine the glycaemic index (GI) testing of whole and ground milled flaxseed muffins as well as the GI of a control muffin (no flaxseed). The GI is the degree to which a food or drink raises the blood glucose level of a person compared with a standard food (glucose drink). Low GI foods will raise blood glucose levels more slowly than high GI foods and foods with a lower GI may help people control their hunger, their appetite, their blood sugar levels and possibly their weight. The Functional Food Centre is internationally recognised as being a leading centre for research into glycaemic response and we continue to be one of the largest centres in Europe working in this area. Through our research we have a large and growing database that enables us to analyse how people's blood glucose responds to different ingredients in foods and drinks and to identify those ingredients that help to better control blood glucose levels.

Why have I been invited to participate?

We are inviting healthy volunteers:

- Aged 18-60 years
- Body mass index (BMI) $\leq 30\text{kg/m}^2$ (BMI means Body Mass Index - we will measure this for you)
- Non-pregnant and non-lactating
- No known diabetes or impaired glucose tolerance
- No medical condition(s) or medication(s) known to affect glucose regulation or appetite and/or which influence digestion and absorption of nutrients
- No major medical or surgical event requiring hospitalisation within the preceding three months
- No use of steroids, protease inhibitors or antipsychotics
- No food allergy to eggs or milk
- Do not use steroids, protease inhibitors or antipsychotics.

Should you agree to participate, and after signing the consent form, you will be asked to complete a health questionnaire to make sure that you are eligible to participate in the study. This information has been provided to help you understand what is involved if you do decide to participate.

What the study involves

If you are eligible and you do decide to take part, then is what the study will involve:

- You will be required to attend a total of six test sessions.
- Each test session will last for approximately 2 to 3 hours. You can decide how many test sessions you do in a week – it could be 1, 2 or 3, but you need to leave at least 1 day between tests
- On your first visit you will be asked to sign a consent form and fill in a health questionnaire. We will also measure your:
 - Height and weight (in order to estimate your body mass index (BMI) – a measure of weight status)
 - Waist circumference.

This study has received clearance from the University Ethics Committee at Oxford Brookes University. If you have any concerns about the conduct of this project, you can contact the Chair of the University Ethics Committee (ethics@brookes.ac.uk).

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- Each test session will involve the following:
 - Two fasting blood glucose measurement (at -5 minutes and 0 minutes) – this involves two small finger-pricks using disposable individual lancets
 - Consumption of the standard or flaxseed/control muffins.
 - Further finger-prick blood samples at 15, 30, 45, 60, 90 and 120 (and 150 & 180 minutes for 3 hour tests) minutes after the start of the meal
 - At the same time intervals, you will be asked how hungry you feel.
- The testing will take place in the GI lab (room S403) for the fasting measurement and the Functional Food Centre kitchen to eat the food. Subsequent measurements will also take place in the lab. You can choose to stay in the lab or come back at the specified time (by special agreement, these measurements can be taken at your office if you are unable to return at the specified times).
- Once you have finished a test you will be offered a snack

What will happen to me if I take part?

In outline the study will involve eating flaxseed/control muffins and having your blood glucose measured over 2 to 3 hours by a trained researcher taking finger prick samples.

How to prepare for the study

- You need to fast overnight (approximately 10-12 hours) – this means **no food or drink**, although you are allowed to drink water in moderation
- For example, if you start your test session at 8.30 am, you need to stop eating/drinking (apart from water) at 8.30 pm the previous evening
- On the day before a test
 - limit your caffeine intake (maximum of 2-3 cups of coffee/tea)
 - no alcohol consumption
 - restrict participating in intense physical activity (e.g. long periods at the gym, excessive swimming, running, aerobics)
- On the day of your session, please wear loose, comfortable clothing where outer garments and footwear can easily be removed
- Limit smoking the day before and do not smoke on the morning of the test.

Benefits of the study

- The study will provide valuable information on the value of glucose release and whether flaxseed can stabilise the blood glucose.
- This information will be used to supply details on the GI of foodstuffs in order to select and identify low GI foods for recommending to the public for improved health and well-being
- You will receive information about your own body profile, including anthropometric and body composition measurements
- You will receive £10 of Amazon or book vouchers per test session.

Health and safety

Your comfort and health and safety are of primary concern to us:

- Each measurement requires only a drop of blood, therefore the finger-prick will be small, with minimal discomfort
- Trained personnel will take finger-prick blood samples using standard procedures
- Finger-pricks are made using disposable individual lancets to avoid the possibility of contamination.

Data protection and withdrawal

All information collected about you will be kept strictly confidential (subject to legal limitations).

This study has received clearance from the University Ethics Committee at Oxford Brookes University. If you have any concerns about the conduct of this project, you can contact the Chair of the University Ethics Committee (ethics@brookes.ac.uk).

- All samples and records will be coded and will only be available to the researchers involved in the study; your name will never appear in any published work
- All data will be securely stored at the University for a minimum of 10 years in accordance with the University's policy on academic integrity
- You are free to withdraw from the study at any time, without giving a reason, and to withdraw any unprocessed data previously supplied

What happens if I do want to take part?

If you would like to take part in this research study you can do so by contacting the researcher at the address, phone number or email address given below. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. At least 12 other people will take part in this study.

Participation is completely voluntary. If you decide to take part you are still free to withdraw at any time and without giving a reason. If you are a student by choosing to either take part or not take part in the study or to withdraw at any time will have no impact on your marks, assessments or future studies. If you are a member of staff by choosing to take or not to take part in the study or to withdraw at any time will have no impact on your employment status at the university no details will be recorded for monitoring purposes.

Who is organising and funding the research?

I am conducting the research as a research student in collaboration with Awatif Almeahmadi (a PhD researcher) at Oxford Brookes University under the supervision of Dr. Helen Lightowler at the Department of Sport & Health Sciences at Oxford Brookes University.

Will this study be kept confidential?

All information collected about you will be kept strictly confidential. Your personal data will be in a locked drawer at Oxford Brookes University, and if saved on a computer, will be securely encrypted. Data in paper or electronic form will be de-identified by a code and kept securely for a period of 10 years after the completion of this research project. You may request details about the storage of your personal data at any time. Only researchers directly involved in the study will have access to personal data. The data will be statistically analysed in the UK under the supervision of Dr Helen Lightowler at Oxford Brookes University.

What will happen to the results of the research study?

The results will be presented as a part of PhD thesis and published in peer-reviewed journals and presented at meetings and conferences, however, all data published will be anonymised. Additionally, the data will be incorporated into our larger research database analysing glycaemic response.

In order to measure the GI of a product, data is required from a minimum of 12 people. You will therefore not receive your own GI result once you have completed the study. However, if you wish to be debriefed, you will be able to contact the research team.

Who has reviewed the study?

This research has been approved by the Oxford Brookes University Research Ethics committee Officer on behalf of the Faculty Ethics Committee.

What should I do if I want to take part?

If you want to take part in this study, please contact:

This study has received clearance from the University Ethics Committee at Oxford Brookes University. If you have any concerns about the conduct of this project, you can contact the Chair of the University Ethics Committee (ethics@brookes.ac.uk).

Awatif Almeahmadi
Functional Food Centre
Oxford Brookes University
Gipsy Lane Campus
Oxford
OX3 0BP
Email: 14099105@brookes.ac.uk
Tel: 01865 483283

Contact for Further Information

If you have any questions regarding this study, you can contact either:

Dr. Helen Lightowler
Functional Food Centre
Oxford Brookes University
Gipsy Lane Campus
Oxford
OX3 0BP
Email: hlightowler@brookes.ac.uk

Thank you for your interest in this study and for taking the time to read this information sheet.

Date

5th October 2016

This study has received clearance from the University Ethics Committee at Oxford Brookes University. If you have any concerns about the conduct of this project, you can contact the Chair of the University Ethics Committee (ethics@brookes.ac.uk).

4

Appendix 2 b: Consent form



Consent form

Glycaemic index (GI) testing of foods

Contacts:

Miss Awatif Almeahmadi

Dr. Helen Lightowler, Director of the Functional Food Centre

Functional Food Centre

Department of Sport and Health Sciences

Faculty of Health and Life Sciences

Oxford Brookes University

Gipsy Lane Campus

Oxford OX3 0BP

Tel: 01865 483283 / 01865 483245

Email: 16079391@brookes.ac.uk / 14099105@brookes.ac.uk / hlightowler@brookes.ac.uk /

Please **INITIAL** the appropriate box

	Yes	No
1. I confirm that I have read and understand the information sheet for the above research project.	☐	☐
2. I confirm that I have had the opportunity to ask questions and have received satisfactory answers to all my questions.	☐	☐
3. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving reason, or to withdraw any unprocessed data previously supplied.	☐	☐
4. I understand that confidentiality of information provided can only be protected within the limits of the law.	☐	☐
5. I agree to take part in the above research.	☐	☐

Name of Participant Date
(block capitals)

Signature

Contact number: email:.....

Name of Researcher Date
(block capitals)

Signature

Health Questionnaire

(Please circle as appropriate)

- Are you allergic to any foods? Yes or No
If yes which one(s)? _____
- Do you have a genetic or metabolic disease? Yes or No
- Are you taking any medication? Yes or No
If yes, which one(s)? _____
- Have you undergone any major medical/ surgical event in the last 3 months? Yes or No
- Are you a smoker? Yes or No
If yes, cigarettes/day: _____
- Are you following a special diet? Yes or No
If yes, which one(s)? _____
- Do you tend to restrain your food intake? Yes or No
- Do you exercise or participate in any sports? Yes or No
How often a week? _____ Duration: _____ Intensity: _____
- Are there any foods you dislike? _____

Women only: please answer the following questions:

- Do you use oral contraceptive pills? Yes or No
- If yes, which type of pill? _____
- What is the average length of your menstrual cycle? _____
- Number of days since the end of your last menstruation? _____

Appendix 2 d: Blood glucose collection sheet

Muffins number: ☐ 1 ☐ 2 ☐ 3

Subject:

Date:

Yesterday evening

What do you eat yesterday evening?

What was the quantity of your meal?

☐ Small ☐ medium ☐ large

Did you do any exercise yesterday?

☐ Yes ☐ No

If yes what was the intensity of it?

☐ None ☐ light ☐ medium ☐ strenuous

What was the duration of the exercise?

☐ 20min ☐ 30 min ☐ 1 hour ☐ other

Did you drink coffee yesterday evening?

☐ Yes ☐ No

If yes how many cup?

Alcohol intake

Glycemic respond (glucose level)

Time of sampling (Minutes)	Glucose level (mmol/l)
0	
15	
30	
45	
60	
90	
120	

Appendix 3 a: Ethical approval letter



Dr Helen Lightowler
Director of Studies
Department of Sport and Health Sciences
Faculty of Health and Life Sciences
Oxford Brookes University
Gipsy Lane
Headington

23 November 2015

Dear Dr Lightowler

UREC Registration No: 150959

The effect of flaxseed in different forms (whole and ground) on glycaemic and insulinaemic response

Thank you for your email of 20 October 2015 outlining your response to the points raised in my previous letter about the PhD study of your research student Awatif Almeahmadi and attaching the revised documents. I am pleased to inform you that, on this basis, I have given Chair's Approval for the study to begin.

The UREC approval period for this study is two years from the date of this letter, so 23 November 2017. If you need the approval to be extended please do contact me nearer the time of expiry.

Should the recruitment, methodology or data storage change from your original plans, or should any study participants experience adverse physical, psychological, social, legal or economic effects from the research, please inform me with full details as soon as possible.

Yours sincerely

A handwritten signature in blue ink, appearing to read "S Quinton", with a stylized flourish at the end.

Dr Sarah Quinton
Chair of the University Research Ethics Committee

cc: Catherine Kerr, Supervisory Team
Awatif Almeahmadi, Research Student
Dido Green, Research Ethics Officer
Jill Organ, Research Degrees Team
Louise Wood, UREC Administrator



www.brookes.ac.uk

Information sheet

Glycaemic and Insulinaemic response of whole and ground flaxseed muffins

You are being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take the time to read the following information carefully:

The purpose of the study

Flaxseed is an oilseed which is of interest from both nutritional and medical aspects due to its special chemical composition and bioactive compounds, which can provide health benefits and can prevent diseases such as cardiovascular disease and type 2 diabetes. Flaxseed can be consumed in many different forms as whole flaxseed, ground meal or extracted oil, which makes it an appropriate functional ingredient for inclusion in different products, for example baked cereal products, bread, muffins and spaghetti. This study aims to determine the effect of the different forms of flaxseed on glycaemic and insulinaemic response.

Why have I been invited to participate?

We are inviting healthy volunteers to participate in the study:

- Aged 18-65 years
- Body mass index (BMI) $\leq 30\text{kg/m}^2$
- Fasting blood glucose $< 6.1\text{ mmol/l}$
- Non-pregnant and non-lactating
- No known diabetes or impaired glucose tolerance
- No medical condition(s) or medication(s) known to affect glucose regulation or appetite and/or which influence digestion and absorption of nutrients
- No major medical or surgical event requiring hospitalisation within the preceding three months
- No use of steroids, protease inhibitors or antipsychotics
- No food allergy to eggs or milk

Should you agree to participate, and after signing the consent form, you will be asked to complete a health questionnaire to make sure that you are eligible to participate in the study.

What the study involves

- Each test session will last for approximately 2.5 hours. You need to attend three test sessions, with at least one day between the tests
- On your first visit we will also measure your:
 - Height and weight (in order to estimate your body mass index (BMI), a measure of weight status)
 - Blood pressure
 - Waist circumference
- After taking the measurements and signing the consent form, you will complete the first test session, which will involve the following:

- Two fasting blood glucose measurement (at 5 minutes and 0 minutes before the meal) – this involves two small finger-pricks using disposable individual lancets
- Further finger-prick blood samples at 15, 30, 45, 60, 90 and 120 minutes after the start of the meal
- At the same time intervals, you will be asked to rate how hungry you feel
- You need to attend the Functional Food Centre (FFC) for the fasting blood measurements and to eat the food. For the subsequent measurements you can either remain in the FFC or come back at the relevant times

How to prepare for the study

- You need to fast overnight (approximately 10-12 hours) – this means **no food or drink**, apart from drinking water in moderation
- For example, if you start your test session at 8.30 am, you need to stop eating/drinking (apart from water) at 8.30 pm the previous evening
- On the day before a test
 - limit your caffeine intake (maximum of 2-3 cups of coffee/tea)
 - limit your alcohol intake (maximum of 1 glass of wine/1 pint of beer)
 - restrict participation in intense physical activity (e.g. long periods at the gym, excessive swimming, running, aerobics)
- Do not smoke on the morning of the test

Benefits of the study

- The study will provide valuable information on the glucose release and insulin response to flaxseed muffins
- You will receive a “health check” profile, including anthropometric and body composition measurements
- You will receive a £30 Amazon voucher on completion of the study

Health and safety issues

- Each measurement requires a small amount of blood; therefore the finger-prick will be small, with minimal discomfort. A light bruising may also occur in some people, but this should disappear within a couple of days and will not affect your ability to work
- Trained personnel will take finger-prick blood samples using standard procedures
- Finger-pricks are made using disposable individual lancets to avoid the possibility of contamination

Data protection and withdrawal

- Confidentiality of any information provided can only be protected within the limitations of the law
- All samples and records will be coded and will only be available to the researchers involved in the study; your name will never appear in any published work
- All data from the study will be owned by Oxford Brookes University and will be stored at the University for a minimum of 10 years
- You are free to withdraw from the study at any time, without giving a reason, and to withdraw any unprocessed data previously supplied

What happens if I do want to take part?

If you would like to take part in this research study you can do so by contacting the researcher at the address, phone number or email address given below. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form.

Participation is completely voluntary. If you decide to take part you are still free to withdraw at any time and without giving a reason. If you are a student, by choosing to either take part or not take part in the study or to withdraw at any time will have no impact on your marks, assessments or future studies. If you are a member of staff by choosing to take part, or not to take part, in the study or to withdraw at any time will have no impact on your employment status at the university, no details will be recorded for monitoring purposes.

Who is organising and funding the research?

I (Awatif Almeahmadi) am conducting the research as a PhD researcher at Oxford Brookes University under the supervision of Dr Helen Lightowler and Dr Catherine Kerr in the Department of Sport & Health Sciences. This research is being funded by the Royal Embassy of Saudi Arabia.

Will this study be kept confidential?

All information collected about you will be kept strictly confidential. Your personal data will be in a locked drawer at Oxford Brookes University, and if saved on a computer, will be securely encrypted. Data in paper or electronic form will be de-identified by a code and kept securely for a period of 10 years after the completion of this research project. You may request details about the storage of your personal data at any time. Only researchers directly involved in the study will have access to personal data. The data will be statistically analysed in the UK under supervision of Dr Helen Lightowler and Dr Catherine Kerr at Oxford Brookes University.

What will happen to the results of this research study?

The results will be presented as a part of a PhD thesis and in scientific journals; however, all data published will be anonymised.

Who has reviewed the study?

This study has been approved by Oxford Brookes University Research Ethics Committee (UREC). Any concerns about the conduct of the study should be referred to the Chair of UREC on ethics@brookes.ac.uk.

Contact for further information

You can contact the researchers at any time if you have any questions or concerns:

Awatif Almeahmadi	14099105@brookes.ac.uk	Tel: 01865 483283
Dr Helen Lightowler	hlightowler@brookes.ac.uk	Tel: 01865 483245
Dr Catherine Kerr	catherine.kerr@brookes.ac.uk	Tel: 01865 484332

Thank you for taking your time to read this information sheet.

Consent form

The effect of flaxseed in different forms (whole and ground) on glycaemic and insulinaemic response

Contacts:

Dr Helen Lightowler	hlightowler@brookes.ac.uk	Tel: 01865 483245
Dr. Miriam Clegg	mclegg@brookes.ac.uk	Tel: 01865 484332
Dr. Magali Chohan	magalichohan@brookes.ac.uk	Tel: 01865 483956
Awatif Almeahmadi	14099105@brookes.ac.uk	Tel: 01865 483283

Functional Food Centre
Department of Sport and Health Sciences
Oxford Brookes University
Gipsy Lane Campus
Oxford OX3 0BP
Tel: 01865 483818 / 01865 483988 / 01865 484365
Email: hlightowler@brookes.ac.uk / catherine.kerr@brookes.ac.uk / 14099105@brookes.ac.uk

Please **INITIAL** the appropriate box

	Yes	No
1. I confirm that I have read and understand the information sheet for the above research project.	☐	☐
2. I confirm that I have had the opportunity to ask questions and have received satisfactory answers to all my questions.	☐	☐
3. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving reason, and to request for any unprocessed data previously supplied to be withdrawn.	☐	☐
4. I understand that confidentiality of information provided can only be protected within the limits of the law.	☐	☐
5. I agree to take part in the above research.	☐	☐

Name of Participant Date
(block capitals)

Signature

Contact number: email:

Name of Researcher Date
(block capitals)

Signature

Appendix 3 d: Satiety / hunger scale

Satiety/hunger scales.

0 mins



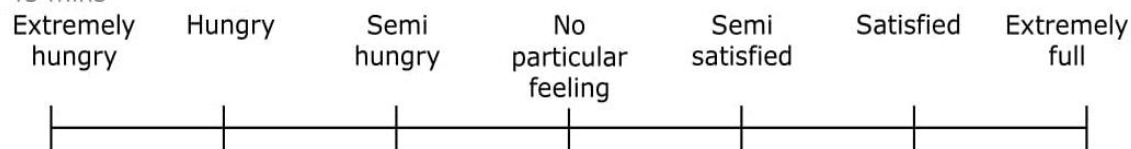
15 mins



30 mins



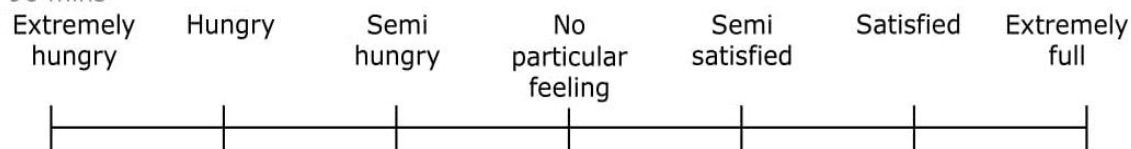
45 mins



60 mins



90 mins



120 mins



Appendix 3 e: Sensory evaluation form scale

Palatability Scales (Sensory evaluation)

Muffins number: 1 2 x3

Subject: _____ Date: / /

Appearance



Colour



Flavour



Texture



Overall acceptability



Appendix 3 f: Gastrointestinal symptom questionnaire

Subject: Muffins: 1 2 3

During the last 24 hour have experienced any of these symptoms:

	None	Moderate	Quite a lot	Sever	Unbearable
Abdominal pain					
Epigastric pain					
Heartburn					
Regurgitation					
Abdominal rumbling					
Belching					
Empty Feeling					
Nauseas					
Vomiting					
Loss of appetite					
Postprandial fullness					
Flatulence					
Diarrhea					
Constipation					

Appendix 4 a: Ethical approval letter



Dr Helen Lightowler
Director of Studies
Department of Sport and Health Sciences
Faculty of Health and Life Sciences
Oxford Brookes University
Headington Campus

17 January 2017

Dear Dr Lightowler

UREC Registration No: 161046 (Resubmission)
The effect of split dose of flaxseed on glycaemic response over 24 hours

Thank you for your email of 13 January outlining your response to the points raised in my previous letter about the PhD study of your research student Awatif Almeahmadi and attaching the revised documents. I am pleased to inform you that, on this basis, I have given Chair's Approval for the study to begin.

The UREC approval period for this study is two years from the date of this letter, so 17 January 2019. If you need the approval to be extended please do contact me nearer the time of expiry.

Should the recruitment, methodology or data storage change from your original plans, or should any study participants experience adverse physical, psychological, social, legal or economic effects from the research, please inform me with full details as soon as possible.

Yours sincerely

A handwritten signature in blue ink, appearing to read "S Quinton", with a long horizontal flourish extending to the right.

Dr Sarah Quinton
Chair of the University Research Ethics Committee

cc Miriam Clegg and Magali Chohan, Supervisory Team
Awatif Almeahmadi, Research Student
Anne Delextrat, Research Ethics Officer
Jill Organ, Research Degrees Team
Louise Wood, UREC Administrator



www.brookes.ac.uk

Participant Information Sheet

Effect of a split dose of flaxseed on 24-hour glucose response

You are invited to take part in a research study. Before you decide, it is important for you to understand why this research is being carried out and what it will involve. Please take the time to read the following information carefully.

The purpose of the study

Flaxseed is an oilseed which is of interest from both nutritional and medical aspects, due to its special chemical composition and bioactive compounds. These compounds may provide health benefits and prevent diseases, such as cardiovascular disease and type 2 diabetes. Flaxseed can be consumed in many different forms as whole flaxseed, ground meal or extracted oil, making it an appropriate functional ingredient for inclusion in many products, such as baked cereal products, bread, muffins and spaghetti.

This study aims to determine the effect of using one or 3 doses of flaxseed on blood glucose response over 24 hours by using a continuous glucose monitoring system (CGMS). The device will be worn over a 24 hour period, to determine whether flaxseed is more effective in terms of blood glucose when consumed once, or split into three times per day.

Blood glucose levels will also be monitored before and after consuming the flaxseed muffins; the meals and flaxseed muffins will be consumed at specific times during the day so the effect of these on blood glucose can be seen.

How will the test be carried out?

Blood glucose levels will be measured using a device with a sensor to monitor glucose. The tiny single-use sensor is introduced under the skin of your abdomen using an automatic insertion device. Insertion is quick and virtually painless. The sensor sits just beneath the skin, and you should not feel it at all. Trained personnel will perform the insertion.

The sensor is secured with tape to keep it in place. You wear the sensor throughout your normal activities from 3 pm the day before the study day, until 9 am the day after the study day. The sensor will be inserted in a different place each test session. The sensor is then removed and discarded, whilst the information collected on the monitor is downloaded onto a computer programme for analysis.

Why have I been invited to participate?

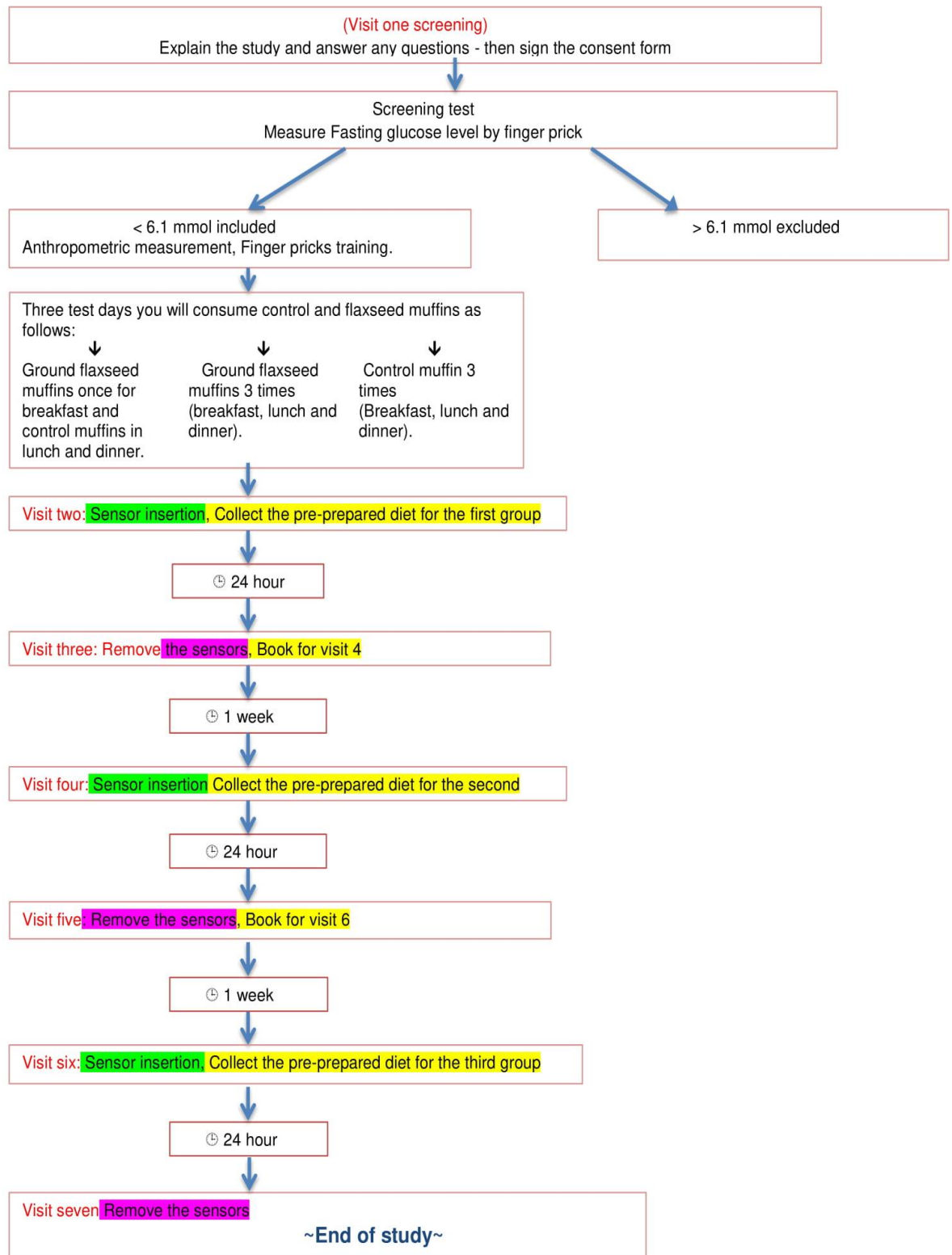
We are inviting healthy volunteers:

- Aged 18-65 years
- Body mass index (BMI) $\leq 30\text{kg/m}^2$
- Fasting blood glucose $< 6.1\text{ mmol/l}$
- Non-pregnant and non-lactating
- No known diabetes or impaired glucose tolerance
- No medical condition(s) or medication(s) known to affect glucose regulation or appetite and/or which influence digestion and absorption of nutrients
- No major medical or surgical event requiring hospitalisation within the preceding three months
- No use of steroids, protease inhibitors or antipsychotics
- No food allergy to eggs or milk
- No known allergies to flaxseed

Should you agree to participate, and after signing the consent form, you will be asked to complete a health questionnaire to make sure that you are eligible to participate in the study.

What the study involves

You will be required need to attend for seven visits, as detailed below:



If you meet the inclusion criteria and agree to participate, you will be provided with an evening meal for the day before each test session as well as meals for each test day (breakfast, lunch, snack and dinner). These will include ready-to-eat meals (for example vegetable pasta), sandwiches (for example tuna), salad and fruits. Flaxseed and control muffins will be provided as a part of the diet. There will be a washout period of one week between the tests.

How to prepare for the screening visit:

- You need to fast overnight (approximately 10-12 hours) – this means **no food or drink**, although you are allowed to drink water in moderation
- For example, if you start your test session at 8.30 am, you need to stop eating/drinking (apart from water) at 8.30 pm the previous evening
- Do not smoke on the morning of the test

This is for the first visit only, the rest of the test sessions you do not need to be fasted.

On your first visit we will also measure your:

- Height and weight (in order to estimate your body mass index (BMI) – a measure of weight status)
- After taking the measurements and signing the consent form you will do the first test session, which will involve the following:
 - Two fasting blood glucose measurement (at -5 minutes and 0 minutes) – this involves two small finger-pricks using disposable individual lancets.

On each day of the study, you will be instructed to take and record seven capillary finger-prick blood glucose measurements (before and after each main meal and at bedtime) using a single-use lancing device to standardize the sensor. You will be fully trained by the researcher about the finger-prick method so you can do it by yourself and will be provided with a blood glucose meter and lancets.

This session will take approximately one hour.

What will I have to do if I do take part?

On the day prior to the study day you will be required to:

- Attend the Functional Food Centre at 3-4 pm for insertion of the sensor. You can ask us any questions at this time. You will need to remain in the department for 1 hour to allow the sensor to initialise.
- Eat the evening meal provided for you. This meal will be given to you to take home.
- Take two finger-prick blood glucose readings for the purpose of calibrating the monitor before dinner and before bed. These blood samples are small and cause minimal discomfort.

- Avoid alcohol whilst the sensor is in position.
- Restrict intense physical activity (e.g. long periods at the gym, running, aerobics).
- There are no restrictions in the types of clothes that you wear.
- You can take shower while you have the sensor but you need to avoid immersion in hot water, as it will affect the sensors.
- You will be provided with the diet for your test day.

This session will take approximately one and half-hours

On the study day you will:

- Be required to have breakfast on 8.00 - 8.30am.
- Have your lunch between 12.30 - 1.30pm
- Have your dinner from 7-8 pm.
- Be required to take 6 further finger-prick blood glucose readings at set times throughout the day.
- Be asked to complete questionnaires on your feelings of hunger and fullness.

NB: We request that you have nothing else to eat or drink during this day other than the food and snacks provided.

On the day after the study day you will:

- Attend the functional food centre for removal of the sensor before you have anything to eat that day (approx 9am).
- Record your total food intake for the day, we will provide you with a food diary for this.
- Book for your next visit.

This session will take approximately half-hour.

Benefits of the study

- The study will provide valuable information on the glucose release and whether flaxseed can stabilise the blood glucose during the day, which would be beneficial for people with type 2 diabetes.
- You will receive a “health check” profile, including anthropometric and body composition measurements.

You will receive £50 of Amazon voucher when you have completed all the sessions

Health and safety issues

- Each finger-prick measurement requires a small amount of blood; therefore the finger-prick will be small, with minimal discomfort. A light bruising may also occur in some people, but this should disappear itself within a couple of days and will not affect your ability to work.
- Trained personnel will take finger-prick blood samples using standard procedures.
- Finger-pricks are made using disposable individual lancets to avoid the possibility of contamination.
- After insertion of the sensor it should be checked regularly to ensure it remains in place and that there is no local redness, tenderness or irritation.
- Trained personnel will insert the sensor.

You can contact the main researcher (Awatif Almeahmadi) on the mobile phone number below if you have any problems with the sensor. In case of emergency, you can call the helpline (01923 205167) of the sensor company which offers support 24/7.

Data protection and withdrawal

- Confidentiality of information provided can only be protected within the limitations of the law.
- All samples and records will be coded and will only be available to the researchers involved in the study; your name will never appear in any published work.
- All data from the study will be owned by Oxford Brookes University and will be stored at the University for a minimum of 10 years.

- You are free to withdraw from the study at any time, without giving a reason, and to withdraw any unprocessed data previously supplied.

What happens if I do want to take part?

If you would like to take part in this research study you can do so by contacting the researcher at the address, phone number or email address given below. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form.

Participation is completely voluntary. If you decide to take part you are still free to withdraw at any time and without giving a reason. If you are a student by choosing to either take part or not take part in the study or to withdraw at any time will have no impact on your marks, assessments or future studies. If you are a member of staff by choosing to take or not to take part in the study or to withdraw at any time will have no impact on your employment status at the university no details will be recorded for monitoring purposes.

Who is organising and funding the research?

I am conducting the research as a PhD researcher at Oxford Brookes University under the supervision of Dr Helen Lightowler, Dr Miriam Clegg and Dr Magali Chohan at the Department of Sport & Health Sciences at Oxford Brookes University. This research is being funded by the Royal Embassy of Saudi Arabia.

Will this study be kept confidential?

All information collected about you will be kept strictly confidential. Your personal data will be in a locked drawer at Oxford Brookes University, and if saved on a computer, will be securely coded. Data in paper or electronic form will be de-identified by a code and kept securely for a period of 10 years after the completion of this research project. You may request details about the storage of your personal data at any time. Only researchers directly involved in the study will have access to personal data. The data will be statistically analysed in the UK under supervision of Dr Helen Lightowler, Dr Miriam Clegg and Dr Magali Chohan at Oxford Brookes University.

What will happen to the results of this research study?

The results will be presented as a part of a PhD thesis and in scientific journals; however, all data published will be anonymised. If you wish to obtain a copy of the published research you can do so by contacting the researchers at the address, phone number or email address given below following completion of the study. Furthermore, a summary of your personal results will be available to you, if interested.

Who has reviewed the study?

This study has been approved by the Oxford Brookes University Research Ethics committee Officer on behalf of the Faculty Ethics Committee. If you have any concerns about the conduct of the study should be referred to the chair of UREC on ethics@brookes.ac.uk

Contact for further information

You can contact the researchers at any time if you have any questions or concerns

- | | | |
|------------------------|--|--|
| • Awatif Almeahmadi | 14099105@brookes.ac.uk | Tel: 01865 483283
Mobile: 07956114474 |
| • Dr. Helen Lightowler | hlightowler@brookes.ac.uk | Tel: 01865 483245 |
| • Dr. Miriam Clegg | mclegg@brookes.ac.uk | Tel: 01865 483245 |
| • Dr. Magali Chohan | magalichohan@brookes.ac.uk | Tel: 01865 483956 |

Thank you for your time to read the information sheet

Appendix 4 c: Consent form

Consent form

Effect of a split portion of flaxseed on 24-hour glucose response

Contacts:

Awatif Almeshmadi 14099105@brookes.ac.uk Tel: 01865 483283

Dr. Helen Lightowler hlightowler@brookes.ac.uk Tel: 01865 483245

Dr. Miriam Clegg mclegg@brookes.ac.uk Tel: 01865 483245

Dr. Magali Chohan magalichohan@brookes.ac.uk Tel: 01865 483956

Functional Food Centre

Department of Sport and Health Sciences

Oxford Brookes University

Gipsy Lane Campus

Oxford OX3 0BP

Please **INITIAL** the appropriate box

	Yes	No
1. I confirm that I have read and understand the information sheet for the above research project.	☐	☐
2. I confirm that I have had the opportunity to ask questions and have received satisfactory answers to all my questions.	☐	☐
3. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving reason, and to request for any unprocessed data previously supplied to be withdrawn.	☐	☐
4. I understand that confidentiality of information provided can only be protected within the limits of the law.	☐	☐
5. I agree to take part in the above research.	☐	☐

Name of Participant (block capitals) Date

Signature

Contact number:
Name of Researcher
(block capitals)

email:
Date

Signature

Please Read Instructions

Simple tips, instructions and guidelines for iPro2 use

On the day of inserting the sensor

- Take your first blood glucose BG meter reading at least 1 hour after the insertion of the sensor.
- Take a second BG meter reading at least 2 hours after the insertion of the sensor.
- Collect third BG meter reading before dinner.
- Collect the last BG meter reading before going to bed.
- Put the used lancets after each blood sample in the sharp bin which provided.

On the test day

- Collect at least 4 BG meter readings each day, before breakfast, lunch, dinner, and bedtime.
- Do not change any settings on your meter during the study, even if a daylight savings time change occurs.
- Put the used lancets after each blood sample in the sharp bin which provided.
- Use the same blood glucose meter for all BG meter readings.
- Do not let anyone else use your meter during the study.

Log sheet entries

- Write down your BG meter readings, physical activity and other events (such as feeling hypoglycemic, stress, or illness).
- Keep the log sheet with you at all times so you can write down the information immediately after each event.
- Record the time and date within 5 minutes of each BG meter reading.

Care and wearing

- Avoid alcohol whilst the sensor is in position.
- Live your life as you normally would. If you normally exercise, then exercise. However, you need to restrict intense physical activity (e.g. long periods at the gym, running, aerobics).
- Keep tape over the sensor and iPro2 to prevent accidental removal or sensor movement. If the sensor comes out even a small amount, it may stop working. If new tape is needed, just put it over the existing tape. If the sensor comes out, place the sensor and iPro2 into a plastic resealable bag and notify the researcher.
- Check the site 4 times a day to ensure that the sensor and iPro2 are firmly connected, the sensor is still fully inserted, and there is no bleeding or irritation.
 - If the sensor is partly pulled out, attempt to gently push it back into place.
 - Remove the sensor if you have redness, pain, tenderness, or swelling at the site, and notify the researcher.
- You may shower and swim while wearing the iPro2 and sensor. The iPro2 is watertight at a depth of up to 2.4 meters (8 feet) for 30 minutes. There is no time limit if you are swimming on the surface of a pool or showering.
- The iPro2 must be removed (but the sensor can be left in) prior to an x-ray, CT scan or MRI. Simply reconnect the iPro2 afterward.

Appendix 4 e: Log sheet for blood glucose

Log sheet for blood glucose

- Please keep the log sheet with you at all times so you can write down the information immediately after each event.
- Record the time and date within 5 minutes of each BG meter reading.
- Put the used lancets after each blood sample in the sharps bin which was provided to you. THANK YOU

Participant code: 12

Date:

Test no: 3

Day 0 Wed 23/8/17	time	Blood glucose
One hour after the sensor insertion		
Two hours after the sensor insertion		
Before dinner		
Before bedtime		
Day 1 Thu 24/8/17		
Before breakfast		
Before lunch		
Before dinner		
Before bedtime		
Day 2 Fri 25/8/17		
Before the sensor removal		

Appendix 4 f: Meal log sheet

Please note that you need to eat all the food which is provided and you should not eat any food which is not provided.

You can have as much tea or coffee as you usually would but please record the amount of tea or coffee as well as if you added any milk or sugar.

Thank you.

Participant's code:

Test number: 2

Day0(night before the test day) Day and date	Time	Please tick that you have eaten the food
Dinner	Please do not forget to take finger prick before dinner	
	Please have your dinner between 6-8pm	
1 Vegetable lasagne		
1 orange		
1 Low fat berry medley yoghurt		
Nuts +fruits		
Day 1 (testing day) Day and date:		
Breakfast	Please do not forget to take finger prick before breakfast	
	Please have your breakfast between 7-9am	
1 flaxseed muffins		
1 Tropicana orange juice		
2 control muffins		
1 Greek style yoghurt		
Snack	Please have your snack between 10-11am	
Nature Valley crunchy bars oat and honey		
Lunch	Please do not forget to take finger prick before lunch	
	Please have your lunch between 12-2pm	
	First hour of lunch	
1 Hummus sandwich		
Nuts		
1 Green salad		
1 Mandarins fruit		
	Second hour of lunch	
1 flaxseed muffin		
1 Apple		
Snack	Please have your snack between 4-5 pm	
Grapes		
Dinner	Please do not forget to take finger prick before dinner	
	Please have your dinner between 6-8pm	
1 Vegetable lasagne		
1 orange		
1 Low fat berry medley yoghurt		
1 Control muffin		
Nuts + fruits		

Day	time	Type of tea and amount (Green , black or herbal tea) 1 Cup	Type of coffee and amount (Black coffee, cappuccino or latte) 1 Cup	Type of milk and amount	Type of sugar and amount

Appendix 4 g: An example of diet composition analysis based on having flaxseed muffins once on the morning for a female

Food Name	Quantity	Energy(Kcal)	Carbohydrate	Protein	Fat
Breakfast					
3 flaxseed muffins	228	616	70	14.6	32.8
Tropicana orange juice	250	121.893	25	1.6	2
Tesco Fat Free Greek Style Yoghurt	100	55	5.3	7.3	0.4
	Meal Total	792.893	100.3	23.5	35.2
Snack 1	21	Meal Total	31.5	1.7	3.6
Lunch					
Nature Valley Crunchy Bars Oat And Honey					
hummus, sandwiches		300	44.7	10.8	6
Apple	152	78	17.6	0.9	0.8
Control Muffins	76	189	31.2	3.6	6.5
nuts only	10	33.4	0.6	1.44	3.4
Tesco Simple Salad	135	36	5.2	1.5	0.6
	Meal Total	636.4	99.3	18.24	17.3
Snack 2 Grapes	35	22.3	5.6	0.2	0.1
Dinner					
Vegetable lasagne	255	350	42	14	14
Control Muffins	76	189	31.2	3.6	6.5
Citrus orange fruit	70	28.7	6.4	0.5	0.1
Tesco Low Fat Berry Medley Yoghurt	125	105	16.4	2.6	3.9
	Meal Total	672.7	96	20.7	24.5
	Total	2220.2	59.9	11.5	32.7

Appendix 4 h: Log sheet for physical activity

Log sheet for physical activities

Participant code: 12

Date:

Test no: 3

Days Wed 23/8/17	Time	Description of Activity	Type and Intensity
Day 0			
Day 1 Thu 24/8/17			

Gastrointestinal Symptom Questionnaire

Subject Id: _____

Study Visit Number _____

Study Visit date: _____

PART 1: Please rate the first stool you pass after this visit as 1-5 on the scale below.

Stool consistency: 1: watery, 2: loose/mushy, 3: soft, formed, 4: formed, 5: hard

1. 2. 3. 4. 5.

PART 2 : Please rate each symptom below by placing a perpendicular slash on the scale as shown in the below. For example, if you experience no side effects and feel the same as normal then a perpendicular line is drawn on the left as below:

The line should reflect any symptoms experienced for the first 24 hours after this study visit.

Nausea (an unpleasant sensation, vaguely referred to the stomach and abdomen, characterised by a feeling of the need to vomit)

Frequency	0 _____ 10
	(absent) (more than usual)
Intensity	0 _____ 10
	(usual) (severe)

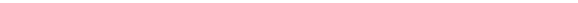
Abdominal cramping (a sudden, involuntary, painful spasmodic muscular contraction)

Frequency	0 _____ 10
	(absent) (more than usual)
Intensity	0 _____ 10
	(usual) (severe)

Distension (the state of being distended or enlarged; the presence of excessive amounts of air or gases in the intestine)

Frequency	0 _____ 10
	(absent) (more than usual)
Intensity	0 _____ 10
	(usual) (severe)

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Frequency 0 (absent)  10 (more than usual)

Additional comments

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