THE IMPACT OF MEAL TIMING OF AMINO ACIDS AND CHROMIUM PICOLINATE ON POSTPRANDIAL GLUCOSE AND INSULIN LEVELS

Master thesis by Danny Sebastian Thomas

OCTOBER 25, 2016

The impact of meal timing of amino acids and chromium picolinate on postprandial glucose and insulin levels

Danny Sebastian Thomas

Thesis submitted for the degree of

Master of Science in Food Technology and Nutrition

October 2016

LUND
UNIVERSITY

Food for Health Science Centre

Department of Food Technology, Engineering and Nutrition

Lund University

Main supervisor: Elin Östman, Food for Health Science Centre, Lund University

Assistant supervisor: Kristina Andersson, Aventure AB

Examiner: Yvonne Granfeldt, Department of Food Technology, Engineering and Nutrition, Lund University

Abstract

One of the most pressing present day health concern is the constantly growing increase of diabetes and the metabolic syndrome. In fact the WHO estimates that as much as 300 million individuals could suffer from diabetes by 2025. While genetic factors do contribute to these issues the more common cause is modern day environmental factors like diets rich in fat or highly processed food, low levels or lack of physical activity, increased stress levels and obesity. Since both diabetes and the metabolic syndrome are characterized partly by elevated blood glucose levels and increasing resistance of cells to the action of insulin, it becomes imperative that treatment measures should focus on controlling blood glucose levels and preventing insulin resistance. A healthy diet and exercise routine can be an effective treatment strategy as well as decrease the risk of likelihood of developing these conditions.

When it comes to diet based treatment, it is imperative that the diet must be rich in low GI foods as these foods elicit a low and steady rise in postprandial glucose levels and hence can prevent high postprandial glycaemia. However these foods are not always accessible and may not necessarily be even liked by the consumers. This is particularly true in the case of breakfast meals as a majority of popular breakfast choices like bread and cereal are high GI foods. One alternative is to develop drinks that can counteract the rise in blood glucose levels when consuming these high GI foods. In fact a candidate drink consisting of carbonated water, chromium picolinate and certain amino acids has shown some promising results with regard to this aspect. While most conventional wisdom dictates a ‘‘what not to eat’’ or ‘‘how much’’ approach towards the treatment or prevention of diabetes or metabolic syndrome, more recent studies have shown that improvements in glycaemia can be obtained by simply timing carbohydrate ingestion after protein and vegetable consumption. The aim of this study was to determine if meal timing had any impact on the efficacy of the candidate drink.

The study was a randomized crossover single blind trial with 20 healthy overweight subjects (BMI 27.63 ± 0.536 kg/m^2; mean ± sem, age 33.50 ± 2.65 years; mean ± sem).The test subjects had to consume a standardized test meal along with a placebo or the test drink. Besides this they were also made to drink the placebo and test drink prior to the meal as well as during the meal on separate occasions. Postprandial blood glucose and insulin levels were measured. The test subjects also had to fill in computerized questionnaires regarding their appetite during each visit. These computerized questionnaires were performed on a 100 mm visual analogue scale (VAS) with end points ranging from ‘‘not at all’’ to ‘‘extremely’’

The results showed that consuming the drink prior to the meal as opposed to during the meal results in a much greater postprandial insulin response in the 0-15 min time interval. No significant difference in postprandial glucose levels were observed between any of the drinks. Finally the results from the appetite data analysis also showed no significant differences.

Preface

This master's thesis project has been performed in the field of functional foods at the Food for Health Science Centre, Lund University in collaboration with Aventure AB. This thesis is submitted for the degree of Masters in Science in Food technology and Nutrition.

This thesis would not have gone so smoothly had it not been for the help of the following individuals

First and foremost I would like to express my gratitude to Elin Östman for granting me the opportunity to do this thesis, for guiding me through the entire process, for answering all my questions and finally for her invaluable help on statistical analysis.

I would like to thank Kristina Andersson without whom the trials would not have gone as smoothly as they did. Thank you for all your help throughout the trials.

I would also like to extend my gratitude to Lisbeth Persson for teaching me how to perform an ELISA test and for always answering my questions no matter how silly they seemed.

Last but not the least I would like to thank all my friends and family for their support and encouragement throughout this thesis.

Contents

1 Introduction

One of the most pressing present day health concern is the constantly growing increase of diabetes and the metabolic syndrome. In fact the WHO estimates that as much as 300 million individuals could suffer from diabetes by 2025 but what is even more alarming is probably the increasing number of children suffering from diabetes. While genetic factors do contribute to this issue the more common cause is probably the unhealthy lifestyle habits. These include a sedentary lifestyle and diets rich in highly processed food etc. owing to urbanization. Hence a part of prevention and treatment is thus based on making lifestyle choices like a healthier diet and regular exercise. In fact The US diabetes Prevention Programme found a 58 % reduction in high risk individuals following a lifestyle change (Hussain et al., 2006).

The glycemic index (GI) refers to a concept used to classify carbohydrate rich foods based on the effect they have on the post prandial blood glucose levels. Low GI foods cause a relatively slow rise in blood glucose levels as well lead to smaller maximal peak as compared to high GI foods. In fact there is plenty of data showing the beneficial effects of low GI foods on lipid and glucose metabolism more importantly a diet rich in low GI foods has been shown to reduce the risk of type 2 diabetes, coronary heart disease and even the metabolic syndrome (Augustin et al., 2015).

However choosing low GI foods is not always that simple particularly during breakfast where there is an abundance of meals consisting of high GI products like bread , cereals etc. Hence there is a need for development of functional foods that are either low in GI or can lower postprandial glycemic responses.

That being said a candidate test drink containing certain amino acids and chromium has already been developed and has been shown to lower postprandial glycemic responses by increasing early postprandial insulin responses in some previously conducted studies (A. Forslund et al., unpublished, Svensson et al., unpublished)

2 Objectives

The primary objective of this study was to determine the effect of timing of delivery of the active components of the candidate test drink mentioned above on post prandial glucose and insulin responses.

3 Background

3.1 Diabetes Mellitus

Diabetes Mellitus is the common name for states of impaired ability for the body to utilize circulating glucose as energy. This lead to persistently higher than normal levels of blood glucose. It is often referred to as three types.

Type 1 diabetes mellitus (T1DM) is caused by autoimmune destruction of the insulin producing beta cells thereby preventing the pancreas from producing insulin. Generally the patients need to be supplied with insulin generally in the form of injections (Codario, 2011).T1DM generally occurs at any early age and hence is sometimes referred to as juvenile diabetes(National Institute of Diabetes and Digestive and Kidney Diseases, 2016).

Type 2 diabetes mellitus (T2DM) on other hand generally occurs in middle aged or older individuals and hence is sometimes referred to as adult onset diabetes. However more and more children seem to be developing this condition nowadays. T2DM is much more common than T1DM and accounts for 90 % of the cases (Nolan, 2006). T2DM results owing to unhealthy lifestyle factors like unhealthy diets, lack of physical exercise, obesity and other complex genetic factors (Simpson, Shaw and Zimmet, 2003) though unlike T1DM the onset of T2DM can be prevented or delayed with healthier lifestyle choices like a healthy diet or regular exercise or pharmacological treatment (Hussain et al., 2006).

The last type is referred to as gestational diabetes as it occurs in women who get higher than normal blood glucose levels during pregnancy. Women with gestational diabetes who do not receive proper care can have an adverse effect on the pregnancy like the baby having an increased risk of hypoglycemia, polycythemia, hyperbilirubinemia, respiratory distress syndrome, hypertrophic cardiomyopathia, and hypocalcemia. Generally women who are overweight, have prediabetes or family members who suffer from T2DM are at a higher risk of developing gestational diabetes (Ignell C, 2015). Gestational diabetes disappears once the baby is born.

In 2015 it was estimated that approximately 415 million people suffer from diabetes. In fact nearly 1 in every 11 adults suffers from diabetes and it is estimated by 2040 at least 642 million people will suffer from diabetes or in other words 1 in every 10 adults will suffer from diabetes (International Diabetes Federation, 2016).In Europe alone nearly 59.8 million were estimated to be suffering from diabetes in 2015 (see figure 1 for more details on regional diabetes populations) and had the highest prevalence of children with T1DM.The situation in developing countries is even more dire. The African continent alone is estimated to have more than two thirds of its population suffering from diabetes as undiagnosed. In fact nearly 46.5 % of adults with diabetes are undiagnosed (International Diabetes Federation, 2015). In 2012 an estimated 1.5 million people died owing to diabetes and another 2.2 million died owing to

high blood glucose. In fact the WHO projects that diabetes will be the 7th leading cause of death by 2030(World Health Organization, 2016). In addition to diabetes, the hyperglycemia associated along with it can lead to the development of other adverse effects such as hypertension, blindness, renal failure and increase risk of infections (Codario, 2011).

The global health expenditure on diabetes was estimated to be at least 376 billion USD in 2010 and is estimated to increase to at least 490 billion USD by 2030. The biggest spender contributing to 52.7 % of the global health expenditure is USA while India; the country with the largest population of people affected with diabetes contributes with roughly 1 % of the total global health expenditure highlighting the discrepancies in treatment between the developing and developed countries. Europe contributes to around 10 % of the global health expenditure. In fact France ,Sweden, The Netherlands and Austria are among the top five countries along with USA that spend the most on diabetes with an average of more than 4000 USD per affected person while Korea is estimated to spend the least amount with an expenditure of approximately 20 USD per affected person (Zhang et al., 2010).

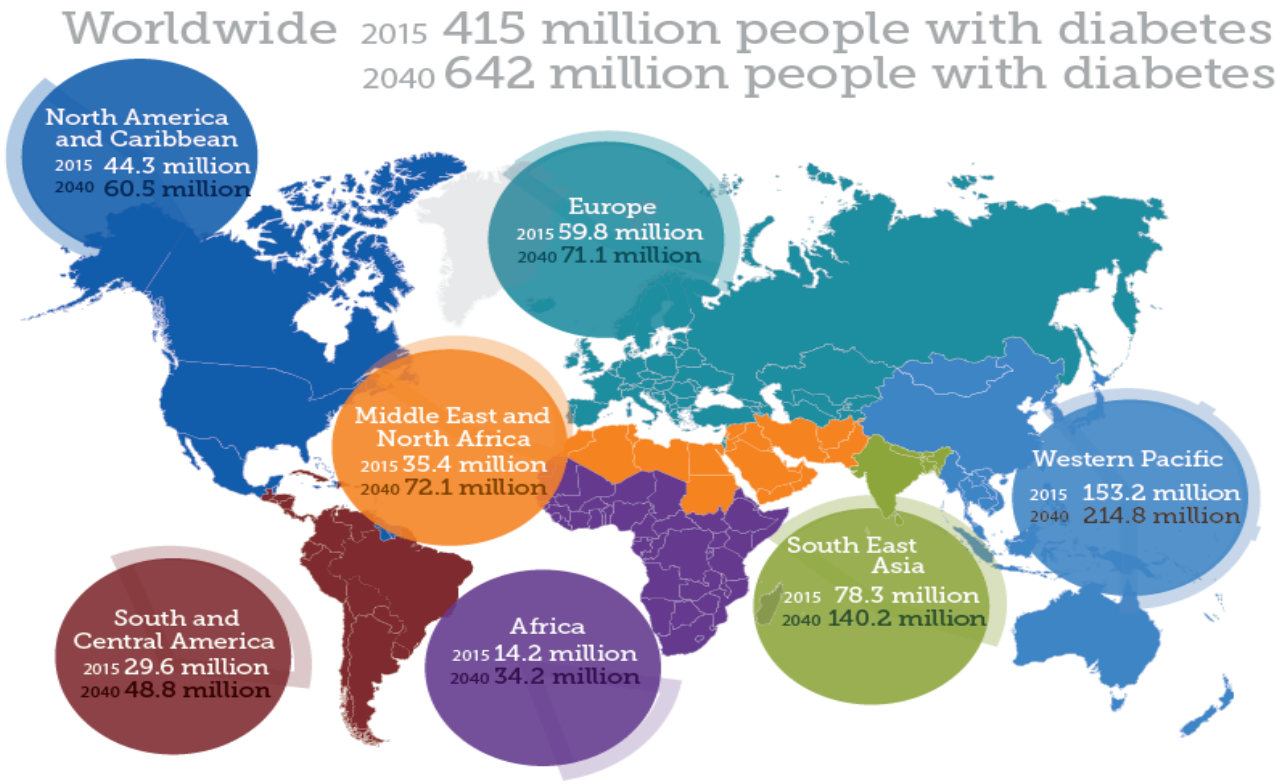

Figure 1- Estimated number of people with diabetes worldwide and per region in 2015 and 2040 (International Diabetes Federation, 2015)

3.2 Metabolic Syndrome

Metabolic Syndrome (MetS) is best defined as an assemblage of several risk factors some of which include hypertension, insulin resistance and obesity. These risk factors in turn lead to higher risk of developing cardiovascular diseases (CVD) and diabetes (Taylor et al., 2013). In fact people with MetS have a fivefold increased risk of developing T2DM. It is estimated

that nearly a quarter of the global adult population suffer from MetS (International Diabetes Federation, 2016).

Since it is a rather complicated topic and the underlying causes for it are not completely understood yet there are multiple definitions but the most common include obesity, elevated blood pressure levels, lowered HDL cholesterol levels, raised blood triglyceride levels and dysglycemia (Prasanna Kumar, 2011). Like T2DM, MetS is strongly influenced by unhealthy lifestyle factors like lack of exercise, smoking or a diet high in highly processed food as well other factors like stress or even genetics (Han and Lean, 2011).

The accumulation of large amounts of intra-abdominal fat is one of the most commonly accepted causes of MetS (Taylor et al., 2013). These fat cells release various pro inflammatory factors like IL-1,IL-6 adipokines etc. which in turn seem to lead to conditions like insulin resistance ,hypertension or even CVD (Han and Lean, 2011, Taylor et al., 2013).

MetS can be managed or even prevented by following lifestyle changes like a healthy diet, regular exercise, abstaining from smoking or by pharmacotherapy (Han and Lean, 2011).

3.3 Glucose Regulation

Once food enters the mouth it is broken down into smaller constituents by chewing and then transferred to the stomach through the esophagus followed by which it is transferred to the intestines. The digestion process can lead to two distinct phases. The first phase referred to as the absorptive phase refers to the nutrients obtained during the digestion process entering into the bloodstream through the gastrointestinal tract. This is followed by the second phase referred to as the post absorptive phase during which the gastrointestinal tract is empty and hence energy must be obtained from the body's various energy stores. While glucose can be directly converted to energy by cells it can on the other hand also be transformed into other forms like glycogen in muscles or triglycerides in adipose tissues and these forms can be stored for later use.

Once the absorptive phase ends there is shift from net synthesis of glucose, amino acids etc. to their catabolism instead. During this phase there is no glucose being absorbed from the gastrointestinal tract but it is imperative that the plasma glucose concentrations do not fall too low as the central nervous system normally uses glucose as its primary energy source. This is achieved by the following two events. The first event is referred to as glycogenolysis and it is essentially the conversion of glycogen into glucose and occurs in the liver. This is generally the first line of defense in maintaining the blood glucose levels. Glycogenolysis can also occur in the muscle cells but unlike the liver the muscle lacks an enzyme necessary for the conversion of glycogen into glucose and hence instead undergoes glycolysis to yield ATP, pyruvate and lactate. The ATP and pyruvate are used directly by the muscle cell whereas lactate on the other hand enters the bloodstream and is transported to the liver where it is converted into glucose which then enters into the bloodstream. The second event is referred to as gluconeogenesis and it refers to the creation of glucose from other precursors like glycerol or amino acids. In this case the triglycerides present in the adipose tissues are broken down into glycerol and fatty acids which are then transferred to the bloodstream by diffusion. The glycerol that reaches the liver is enzymatically converted into glucose which is then released into the bloodstream. Protein can also become a source of glucose although this

generally occurs a few hours into the post absorptive phase. In this case large amounts of protein from muscle and other tissues can be catabolized into amino acids which then travel through blood to the liver where they are converted into glucose through the a-keto pathways followed by which this glucose is released into the bloodstream. It is imperative to note that continued protein loss during a prolonged fast can have very severe effects like disruption of cell function, sickness and ultimately even death.

All these events are mediated by various hormones among which the most important are probably the two pancreatic hormones insulin and glucagon. Besides these epinephrine and cortisol produced by the adrenal glands, growth hormone from the anterior pituitary gland as well as the sympathetic nerves to the liver and adipose tissue play a role as well (Widmaier, Raff and Strang, 2014).

3.4 Insulin and glucose regulation

Insulin is a peptide hormone secreted by clusters of endocrine cells referred to as the ‘‘islets of Langerhans’’ present in the pancreas. There are several variants of these islet cells each of which produces a different hormone. Insulin is secreted by the beta cells while glucagon is secreted by the alpha cells. Insulin is arguably the primary driving force of all metabolic events that occur during the absorptive and post absorptive states and hence is the most important hormone controlling metabolism. In fact its secretion and hence its plasma concentration increase during the absorptive phase and decreases during the post absorptive phase. This is because following a meal blood glucose levels rise which in turn signals the beta cells to secrete insulin which is then released into the blood stream where it exerts an effect on various tissues like that of the liver, skeletal muscles and even adipose tissue(see figure 3 for summary of the various effects of insulin).

The actual mechanism involves the binding of insulin to certain receptors present on the surface of the plasma membrane of the target cells referred to as the insulin receptor (IR). This in turn leads to a signaling cascade that eventually ends with the translocation of glucose transporter proteins known as GLUT-4 to the cell membrane from which they can transport glucose into the cell. Once the glucose starts entering the cells the blood glucose levels start to drop which in turn signals the beta cells to stop secreting insulin (see figure 2 for an overview of insulin signaling pathway). This in turn leads to a lowering of blood insulin levels which in turn causes the amount of GLUT-4 transporters at the cell membrane to decrease (Widmaier, Raff and Strang, 2014).

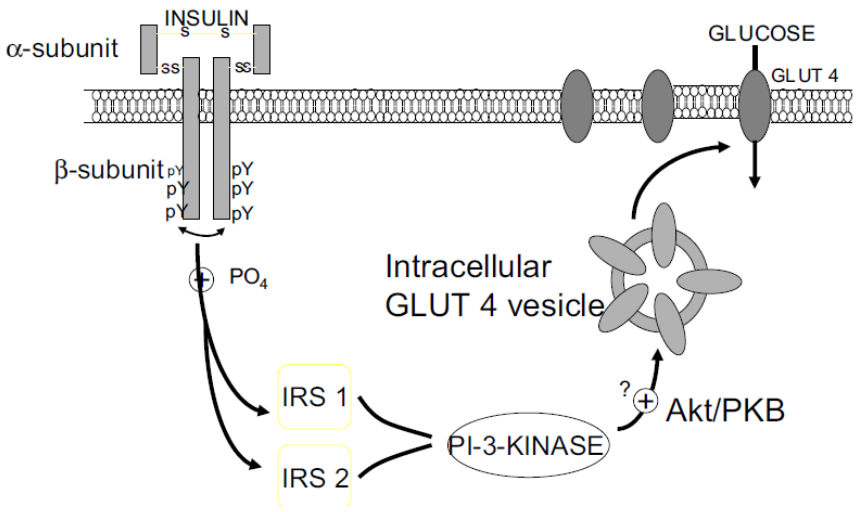

Figure 2 -The insulin signaling pathway (Bhattacharya, Dey and Roy, 2007)

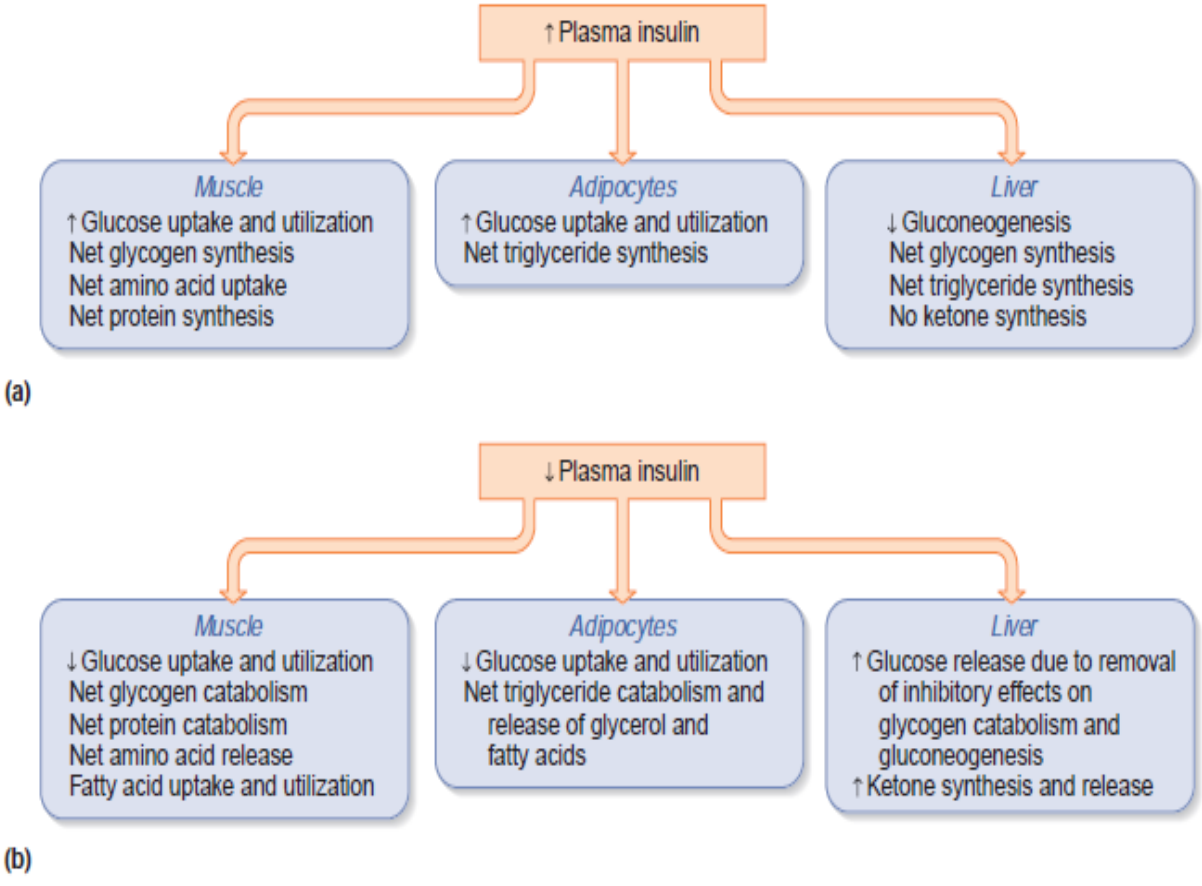

Figure 3 -Summary of overall target cell responses to a) increase or b) decrease of plasma insulin levels (Widmaier, Raff and Strang, 2014).

Besides insulin there are certain other hormones that are released in response to ingestion of a meal referred to as incretins as they are insulinotropic in nature. The most important incretin hormones are glucose dependent insulinotropic polypeptide (GIP) and glucagon like peptide 1 (GLP-1) produced from cells in upper and lower small intestinal mucosa respectively. It has been shown that incretin function is greatly impaired in T2DM as well as any interference with incretin function can cause glucose intolerance. GLP-1 in addition to its insulinotropic effects have also been shown to stimulate new beta cell growth (Holst and Ørskov, 2001). A complete overview of the various insulin stimulating mechanisms can be seen in figure 4.

In addition to incretins insulin secretion is also influenced by various other hormones such as glucagon which is secreted during fasting when glucose levels are low and suppresses insulin secretion. Ghrelin and leptin are two other hormones that have an insulin suppressing effect and that are secreted while fasting (Torres, Noriega and Tovar, 2016).

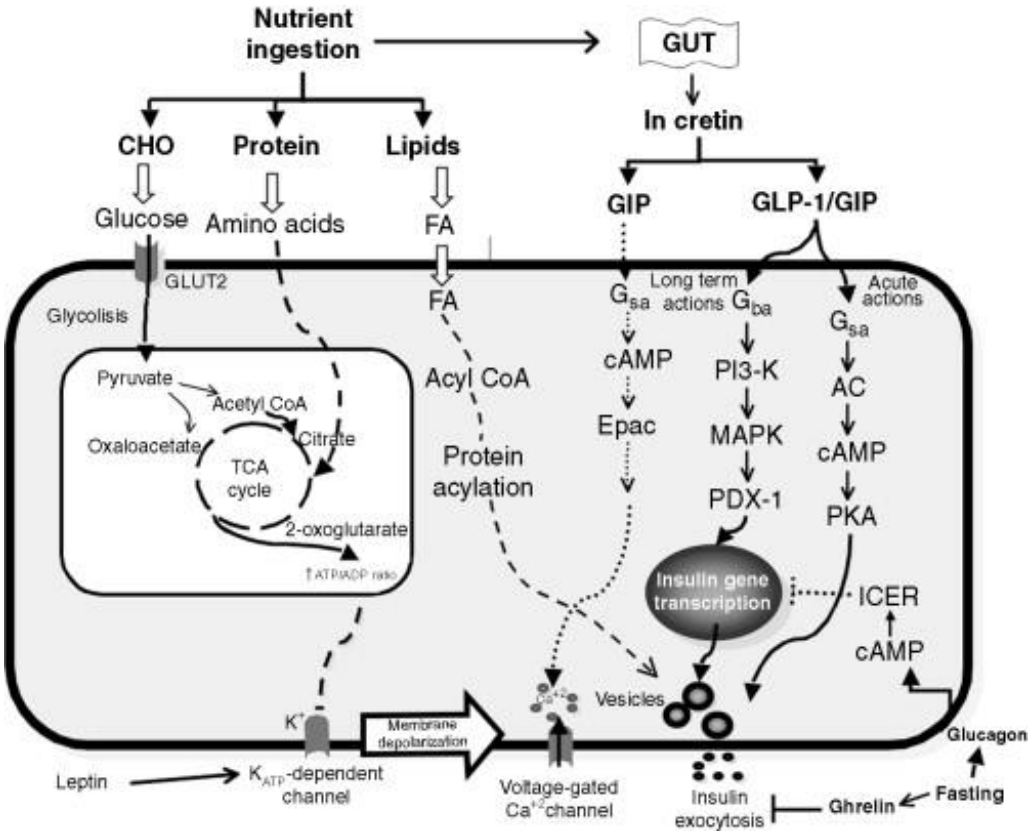

Figure 4 -Overview of the different insulin signaling pathways in the beta cell (Torres, Noriega and Tovar, 2016)

3.5 Role of amino acids in insulin regulation

Aside from glucose, food proteins have also been shown to stimulate insulin response without carbohydrates (Gannon et al., 1992, Saeed et al., 2002).For example whey protein has been

shown to have an insulinotropic effect in both healthy and T2DM patients. More interestingly though is that ingestion of whey along with a high GI meal has been shown to stimulate a much higher insulin secretion as compared to consuming the high GI meal alone while at the same time reducing postprandial blood glucose levels (Frid et al., 2005) which might be a desirable effect. It must be noted however that this increase in insulin levels is desirable provided it occurs during the early window (0-30 mins approximately) of the post prandial phase as this increase as stated above leads to a decrease in blood glucose levels which in turn has been linked to health benefits like reduction in risk of CVD. In fact elevated insulin levels after the postprandial phase could indicate diabetes and can even increase the risk of developing atherosclerosis or coronary heart disease (Kapur et al., 2010). Studies also showed that whey increased GIP levels in the blood but had no effect on GLP-1 levels (Frid et al., 2005). This higher insulin secretion seem to be linked to an increase in serum levels of particular amino acids like leucine, isoleucine, valine, threonine and lysine as well as increased levels of the incretin GIP (Salehi et al., 2012) .In fact another study was able to prove that a mixture of glucose and amino acids leucine, isoleucine, valine, lysine and threonine was shown to mimic the insulinotropic effects of whey with one exception, they had no effect on either incretin hormone (Nilsson, J Holst and ME Björck, 2007).

It has been known for quite some time that when an intravenous mixture of essential amino acids is administered insulin secretion is stimulated (Floyd et al., 1966). The amino acids seem to stimulate insulin secretion by exerting a direct effect on the beta cells of the pancreas (Keane and Newsholme, 2014) though insulin secretion can be stimulated by some amino acids indirectly because of their stimulating effect on the incretin hormones GIP (Lindgren et al., 2015) and GLP-1 (Samocha-Bonet et al., 2015).

3.6 Insulin resistance

As the name implies insulin resistance refers to a decreased response to insulin by the target cells and can occur due to various reasons such as obesity (Lionetti et al., 2009) or certain genetic predispositions (DeFronzo and Ferrannini, 1991) and in turn leads to an increased risk of T2DM (Lionetti et al., 2009).

Generally when a healthy individual starts to consume excessive amounts of calories thereby leading to weight gain, his/her body starts to become less sensitive to the action of insulin and so the beta cells of the pancreas start to produce more insulin which in turn leads to increased plasma insulin levels. However as the weight gain increases , the cells become more and more resistant to the action of insulin and eventually the beta cells of the pancreas reach a point where they simply cannot produce enough insulin to balance the insulin resistance exhibited by the cells. At this point the plasma insulin levels will go back to or below normal followed by which the individual will suffer from severe glucose intolerance. It primarily effects the muscle cells (DeFronzo and Ferrannini, 1991).

Increased levels of free fatty acids (FFA) is thought to be the cause of insulin resistance. Owing to increasing obesity there is an expansion of adipocytes and these stressed adipocytes start to release chemoattractant proteins. They in turn start to attract macrophages around these adipocytes which in turn start to release pro inflammatory cytokines like tumor necrosis factor (TNF) and interleukin 6 (IL-6) and these in turn spread the inflammation to the neighboring adipocytes. These increased levels of inflammation cause insulin resistance which in turn leads to increased levels of free fatty acids in the blood as insulin can no longer exert its

attenuating effect on these FFA effectively anymore. Eventually the adipocytes die from inflammation and release their fat content into the bloodstream. As FFA levels in the blood start to rise it stimulates the further release of pro inflammatory cytokines by interacting with the toll like receptors (TLR-4) present on the cell surfaces (Lionetti et al., 2009, de Luca and Olefsky, 2007).

3.7 Chromium picolinate

Chromium (Cr) is an essential element and plays a role in the metabolism of carbohydrates, lipids, proteins and nucleic acids. It has also been linked to the activity of glucose tolerance factor (GTF) (Huang et al., 2014) and hence its deficiency can lead to impaired glucose tolerance (Brown et al., 1986). The GTF essentially refers to any complex of trivalent chromium since these are considered the biologically active forms. A severe deficiency of Cr can even lead to reversible insulin resistance or even diabetes. (Parsaeyan and Mozaffari–Khosravi, 2012).

Cr has a very poor absorption rate which in turn severely limits its efficacy as supplemental drug. Chromium picolinate (CrPic) on the other hand has a much better absorption rate (0.7-5.2 %) as compared to that of chromium (0.4 – 2 %) thereby making it a much better option as a supplemental drug (Huang et al., 2014). CrPic is essentially a synthetic complex of chromium and picolinic acid and since chromium is trivalent in nature there can be up to three picolinic acid molecules in the complex and it seems that chromium tripicollinate is the most effective (Evans and Pouchnik, 1993). The CrPic molecules are thought to be absorbed by the cells intact where they are proposed to be internalized into the cytoplasm by passive diffusion followed by which they are degraded within 24 hours. Following degradation the chromium is retained in the cells while the picolinate is excreted (Hepburn and Vincent, 2002).

In studies CrPic has been shown to have a positive impact on glycemic levels in T2DM patients (Paiva et al., 2015, Shinde et al., 2004). In fact a meta-analysis conducted of 25 trials showed a positive effect on glycemic control in diabetes patients after chromium supplementation. Additionally chromium supplementation was also shown to improve triglyceride and HDL-C levels (Suksomboon, Poolsup and Yuwanakorn, 2014).

The molecular mechanism behind the action of chromium on glucose metabolism are not fully understood. However studies based on elucidating the action of chromium picolinate show that the complex behaves independently of insulin and instead acts as an insulin analogue. Like insulin, CrPic seems to stimulate the translocation of GLUT-4 receptors from the intracellular components to the cell surface which in turn increases uptake of glucose and amino acids (Paiva et al., 2015, Wang and Yao, 2009). This mechanism for stimulating the increase of GLUT-4 receptors in the plasma membrane seems to be caused by a reduction in plasma cholesterol levels which in turn increases membrane fluidity (Evans and Bowman, 1992, Pattar et al., 2006)

In terms of toxicity, according to the European food Safety Authority (EFSA), the use of CrPic as a source of Cr in supplements or fortified foods is completely safe as long the dose not exceed the amount set originally set by the WHO which is 250 μg Cr per day. EFSA also concluded that CrPic is neither carcinogenic nor does it induce DNA damage(based on in vivo studies) though they expressed concern over the fact that fortified foods and supplement

for Cr must be from Cr(III) source alone and not be contaminated with Cr(VI) which is a proven carcinogen.

3.8 Previous Studies and importance of meal timing

The candidate drink used in this project has been used in some relatively similar versions in previously conducted studies which are mentioned in this section.

In a study conducted by A. Forslund et al (unpublished), the researchers asked their test subjects to consume a carbonated drink after having a high GI meal of white bread, marmalade and butter. The test subjects recruited for the study were overweight but healthy. Each of the test subjects consumed four variants of this drink: one a reference containing flavored carbonated water alone, one a carbonated mixture of water and amino acids (6.9 g), one a carbonated mixture of water and CrPic (500 µg) and finally one containing a mixture of both amino acids (6.9 g) and CrPic (500 µg). They discovered that in both cases there was reduction in post prandial glucose levels following consumption of the meal as compared to the reference drink. More interestingly though they discovered that while both test drinks had higher early postprandial insulin levels (0-15 mins) as compared to the reference this increase was significantly much higher in the drink containing amino acids alone.

There is another study conducted by Svensson et al (unpublished) in which the candidate drink was tested though in this case it was tested more in the form of dose-response manner. The test subjects participating in this study were also healthy but overweight. The test drink in this case had four variants one a placebo and the remaining containing mixtures of amino acids (AA) and CrPic in decreasing amounts. The amounts were 6.9 g AA + 500 µg CrPic, 3.5 g AA + 250 µg CrPic and 1.75 g AA + 125 µg CrPic. The subjects were made to consume part of the drink prior to the meal and the remaining during the meal consisting of mashed potatoes, cod fillet, butter and lingonberry jam. Much like the above mentioned study the researchers found that there was a relatively similar reduction in postprandial glucose levels in all three variants of the test drink. More interestingly though they discovered that the mixture containing the highest dose of the active components had a significantly higher increase in early postprandial insulin levels while the other two doses showed no significant increase as compared to the reference.

While most conventional wisdom dictates a ‘‘what not to eat’’ or ‘‘how much’’ approach towards the treatment or prevention of diabetes, recent studies show that improvements in glycaemia can be obtained by optimal timing of carbohydrates ingestion during a meal. A novel study conducted by Shukla et al in 2015 showed that when test subject ate the protein and vegetable portion of their meal before the carbohydrate portion there was a significant decrease in postprandial glucose and insulin levels as compared to when the same meal was eaten but in the case the carbohydrate portion was eaten first. Another study conducted by Trico et al in 2015 showed a similar result. In this case it was found that small lipid or protein preloads before glucose administration helped reduce post prandial glucose levels in not only healthy individuals but also in patients with T2DM or impaired glucose tolerance. These studies prove the meal order can play an important role in glucose and insulin regulation and hence in turn validates the need for further research in this aspect and is done so in this study.

3.9 Appetite

Sensations of hunger, satiety, desire to eat specific foods and other appetite related sensations are highly subjective and hence can be influenced by a number of factors both internal and external. Internal factors could include physiological and psychological variables while external factors like physical activity, weather, prior meals, temperature etc. can also influence appetite related sensations. This is particularly important when performing appetite related tests as it can affect the reproducibility of these tests (Flint et al., 2000).

Generally tests use to measure subjective appetite sensations are in the form of visual analogue scales (VAS). They consist of lines of varying length with words at each end describing the extremes of the questions being asked. Subjects are asked to make a mark across the line corresponding to their feeling and measurements are then quantified by measuring the distance from the left end of the line to this mark. VAS tests have shown to be quiet effective in terms of reproducibility as well as sensitivity. These test can be performed by hand or on a computer (Flint et al., 2000).

It has been suggested that slow digesting carbohydrates can increase satiety by causing less fluctuations in postprandial glucose levels though results from a meta-analysis of seven studies conducted by Flint et al in 2007 suggested that it was in fact the postprandial insulin levels rather than the post prandial glucose levels that have an effect on satiety. Insulin can affect appetite in multiple ways like stimulation of appetite centers in the hypothalamic area of the brain, interactions with satiety gut hormones like cholecystokinin (CCK), GLP-1 etc. or even by its effects on substrate oxidation in the liver (Flint et al., 2007).

Among the three macronutrients, protein is considered to have the most satiating effect. Various mechanisms have been shown to contribute to protein induced satiety. Like insulin protein too is believed to exert an effect (increase in concentrations) on the various satiety gut peptides like CCK or GLP-1. Besides this protein induced satiety has also been proposed to occur due to its effect on energy expenditure (i.e. diet induced thermogenesis) or also by stimulating gluconeogenesis which in turn improves glucose homeostasis. Certain amino acids have also been shown to contribute to postprandial satiety levels (Veldhorst et al., 2008). It is proposed that certain amino acids like leucine affect appetite by directly stimulating appetite centers in the brain or possibly even act as neurotransmitter precursors (Davidenko et al., 2013).

4 Materials and Methods

4.1 Test meal and drinks.

The test meals always consisted of cod fillets (Findus Torskrygg, Findus AB, Bjuv, Sweden), mashed potatoes (ICA Potatismos, ICA AB, Solna, Sweden), lingonberry jam (Lingonsylt, ICA AB, Solna, Sweden), butter (Smör Normalsaltat, Skånemejerier, Malmö, Sweden) and fresh cucumber. The actual amount of each ingredient were as follows 125 g of cooked cod, 250 g mashed potatoes (44 g powder mixed with 200 ml boiled water), 20 g melted butter, 50 g lingonberry jam and 50 g cucumber(see figure 5 for photo). Each test subject received two versions of the above meal, one containing a carbonated drink that had amino acids and

chromium picolinate (AA + CrPic meal) while the other contained a regular flavored carbonated drink (Ref meal).Bot the test and the reference drinks had a synthetic lemon-lime flavor. In addition to this variation the test subjects were also subject to variation in when the drinks were consumed i.e. half the dose before the meal (PM) with the other half during the meal or the entire dose during the meal (D). In total, each test subject was subjected to a total of four test meals. The macronutrient composition of the meals can be observed in Table 1

Table 1 -Comparison of macronutrient composition of test and reference meals

Macronutrient	AA + CrPic Meal	Ref Meal
Carbohydrate(g)	49	49
Fat(g)	19	19
Protein(g)	34 + 2.6 (AA)	34

Both the reference drinks and the test drinks were stored in plastic bottles with the reference drink having a black cap while the test drinks had a green cap. Both drinks had a total volume of 330 ml. The test drink consisted of a mixture of 2.6 g of amino acids (leucine, isoleucine, valine, threonine, and lysine) and 250 µg of chromium picolinate. All bottles were refrigerated until it was time to serve them. Both the placebo and test drinks were produced at Aqua Nobel In January 2016.

Figure 5- Photo of test meal used in this study

4.2 Study design

The study was a randomized crossover single blind trial. The study included four separate study occasions for each test subject each lasting for approximately 4.5 hours (7:45 am to

12:15 pm). The type of test study being conducted was randomly assigned to each subject with at least a minimum of one week between each test. The test meals were served as breakfast following an overnight fast at around 8 am. The subjects were instructed to finish their meals within 15 minutes and depending on the visit the subjects were instructed to drink the test drink either prior to meal consumption or during the meal. Depending on the timing of the consumption of the drink by the test subjects two different protocols for preparation of the drink were followed. They were

- In the pre meal (PM) test, 165 ml needed to be consumed before the meal and was divided into three cups of approximately 55 ml. They were then served to the test subjects who were instructed to drink them at 3, 5 and 6 minutes respectively before the meal. The remaining 165 ml was divided into five cups of approximately 33 ml and the subjects were instructed to drink these at 1,3,6,9 and 12 minutes after starting the meal
- In the during (D) test 330 ml of the drink was divided into 5 cups of approximately 66 ml and served to the subjects who were instructed to drink them during the 1,3,6,9 and 12 minutes during the meal

A fasting blood sample was collected by a nurse at time 0 i.e. before the test meal was served followed by blood sampling at 15, 30, 45, 60, 90, 120 and 180 minutes following breakfast. Capillary blood samples were used for glucose measurements while a vein catheter was used for insulin measurements at all sample points.

All subjects were instructed to abstain from alcohol and avoid any physical exercise the day before the trial. In addition they were instructed to have a low fiber dinner by around 6 pm followed by a final meal at around 9 or 10 pm consisting of provided white bread (Dollarfranska) along with an optional spread and drink. The subjects were instructed to make a note of the meals they ate at 6 and 9 pm respectively and replicate that exact meal plan before each study visit. The subjects were also allowed to drink up to 200 ml of water the following morning if they woke up thirsty. On the day of the trial the subjects were instructed to choose a mode of transport that required minimal physical activity and were asked to replicate that transport option for their remaining study sessions as well.

4.3 Test Subjects

Twenty healthy and non-smoking subjects consisting of 11 men and 9 women volunteered to participate in the study. Their body mass indices were within the range 23 -31 kg/m^2 (27.63 ± 0.536; mean ± sem) while their ages were within the range 20 -60 years (33.50 ± 2.65; mean ± sem).Their body fat percentage was within the range 15 – 45 %(30.47 ± 1.43; mean ± sem).All subjects also had fasting glucose values well within the normal range (5.5 mmol/L ± 0.053; mean ± sem).

All test subjects volunteered to participate in the study and gave their written informed consent. They were also made aware of the fact that they could withdraw from the study should they choose to do so at any time. The study was approved by the Regional Ethical Review Board at Lund University.

4.4 Blood Analysis

The blood samples obtained for measuring serum insulin were collected in specialized serum separation tubes (BD Microtainer® SST™ Tubes, Becton, Dickinson and Company, Franklin Lakes, NJ, USA).They were allowed to stand at room temperature for 60 mins followed by which they were centrifuged at 21°C for 11 mins at 4000 rpm to separate the serum. The serum was transferred to Eppendorf tubes and stored frozen (-20°C) until the time of analysis. The analysis was made with enzyme immunoassay followed by spectrophotometric detection. The analysis was a semi-automated procedure consisting of an integrated immunoassay analyzer (SPECTROstar-Nano BMG LabTech GmbH, Ortenberg, Germany) and an ELISA kit for insulin (Mercodia insulin ELISA, Mercodia AB, Uppsala, Sweden).

The blood glucose values on the other hand were obtained immediately after sampling using a B-Glucose analyzer (Hemocue, Ängelholm, Sweden).

4.5 Appetite analysis

Each test subject was also given a questionnaire to fill in during each study to describe their food intake and physical activity. Besides this the test subjects also had to fill in computerized questionnaires regarding their appetite and wellbeing as well as one about meal palatability during each visit. These computerized questionnaires were performed on a 100 mm visual analogue scale (VAS) with end points ranging from ‘‘not at all’’ to ‘‘extremely’’. In general the test meals were liked by most subjects with the exception of one subject who found the meal too heavy and another who was allergic to cucumbers. In the first case based on the remainder of the meal left after the first study session the remaining meals were accordingly adjusted and in the second case cucumbers were omitted from the test meal.

4.6 Calculations and statistical methods

The incremental area under curve (iAUC) was calculated for glucose and insulin according to the trapezoidal method excluding areas below the fasting level. The hypothesis that there was no significant difference between the fasting values of insulin and glucose for the different meals was tested using analysis of variance (ANOVA), general linear model, in Minitab (release 17, Minitab Inc., State Collage, PA, USA).Incremental peaks (iPeaks) for glucose and insulin were calculated using GraphPad (GraphPad Prism 7, version 7.01, GraphPad Software, Inc., San Diego, CA, USA).The individual iPeak was defined as the maximal increase in the curve from the baseline over the entire test duration. Glycaemic profile (GP) was calculated for the glucose response and was defined as the duration divided by the iPeak (Rosén et al., 2009). The duration was calculated using GraphPad.

All statistical calculations were performed in Minitab and results were expressed as mean± SEM. To evaluate significant differences for iAUCs of glucose and insulin, as well as iPeaks and GP, general linear model ANOVA was used followed by Tukey’s pair wise comparisons test. For appetite ratings, analysis of variance with covariate (ANCOVA) using total area values and the 0-values as covariate. If the data was not normally distributed (tested with Anderson-Darling test and considered unevenly distributed when $p < 0.05$), it was transformed using a Box-Cox plot prior to ANOVA.

5 Results

5.1 Postprandial glucose response

The delta curves for the postprandial glycemic responses for all four meals are shown in figure 6. The meals were labelled as follows

- **B-D** - Reference drink consumed following the ”during meal” protocol
- **B-PM** - Reference drink consumed following the ”pre meal” protocol
- **G-D** - Test drink consumed following the “during meal” protocol
- **G-PM** - Test drink consumed following the ”pre meal” protocol

The iAUC values were also calculated and are presented in table 2a and 2b while the iPeak and GP values are shown in table 3

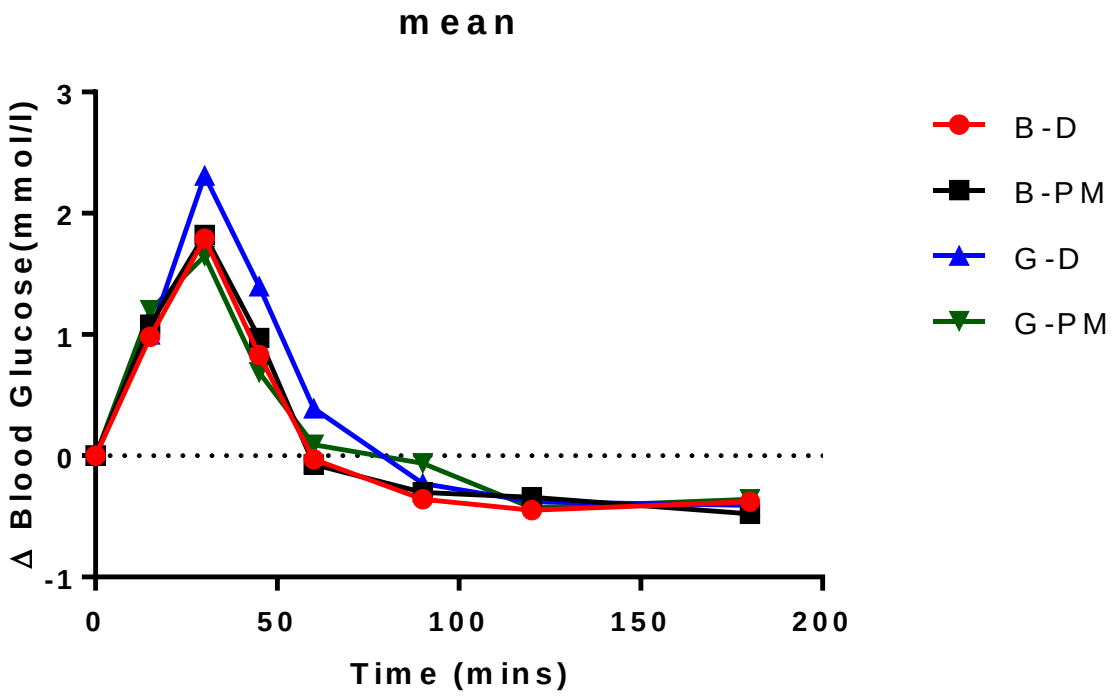

Figure 6 -Glycemic response following intake of the various test meals. Values are expressed as mean± SEM; n=20

Table 2a-Glucose iAUC and change in percentage when compared to reference (0-15, 0-30 and 0-45) (Untransformed mean values are shown) Values in the same column with different superscript letters are significantly different, P < 0.05 (ANOVA followed by Tukey's multiple comparisons test)

Time	**0-15**		**0-30**		**0-45**	
Meal	iAUC (mmol.min/L)	Change (%)	iAUC (mmol.min/L)	Change (%)	iAUC (mmol.min/L)	Change (%)
B-D(Ref)	7.89±1.12[a]	-	28.26±2.73[a]	-	48.00±3.67[a]	-
B-PM	8.80±1.13[a]	+11.53	30.12±2.85[a]	+6.58	51.11±4.51[a]	+6.47
G-D	8.01±1.19[a]	+1.52	32.39±2.82[a]	+14.61	60.19±4.17[a]	+25.39
G-PM	8.96±1.19[a]	+13.56	30.40±3.53[a]	+7.57	49.05±5.79[a]	+2.18

Table 2b -Glucose iAUC and change in percentage when compared to reference (0-120 and 0-180) (Untransformed mean values are shown)

Time	**0-120**		**0-180**	
Meal	iAUC (mmol.min/L)	Change (%)	iAUC (mmol.min/L)	Change (%)
B-D(Ref)	62.94±5.58[a]	-	68.34±7.18[a]	-
B-PM	65.21±6.67[a]	+3.60	67.81±7.45[a]	-0.77
G-D	87.70±8.31[a]	+39.33	93.37±9.39[a]	+36.62
G-PM	68.64±10.30[a]	+9.05	71.30±11.70[a]	+4.27

Table 3-Change in iPeak And GP values (Values expressed as mean±sem)

Meal	**iPeak value (Δmmol/l)**	**Change (%)**	**GP value (Duration/iPeak)**	**Change (%)**
B-D (Ref)	1.79±.119[abc]	-	33.22±4.28[a]	-
B-PM	1.82±.164[abc]	+1.67	32.41±3.67[a]	-2.43
G-D	2.31±.191[b]	+29.05	34.14±3.45[a]	+2.76
G-PM	1.65±.183[c]	-8.28	47.27±8.04[a]	+42.29

As mentioned earlier in the statistical methods and calculations section, a general linear model ANOVA followed by Tukey's Comparision test was used to ascertain any significant differences. These tests were run for all time intervals mentioned in table 2a and 2b and no significant differences were found between the different meal types in any of these time intervals. A significant difference was observed between the iPeak values of G-D and G-PM as seen in table 3.

5.2 Postprandial insulin response

The delta curves for the postprandial insulin responses for all four meals are shown in figure 7. The meals were once again labelled as follows

- **B-D** - Reference drink consumed following the "during meal" protocol
- **B-PM** - Reference drink consumed following the "pre meal" protocol
- **G-D** - Test drink consumed following the "during meal" protocol
- **G-PM** - Test drink consumed following the "pre meal" protocol

The iAUC values were also calculated and are presented in table 4a and 4b while the iPeak values are shown in table 5

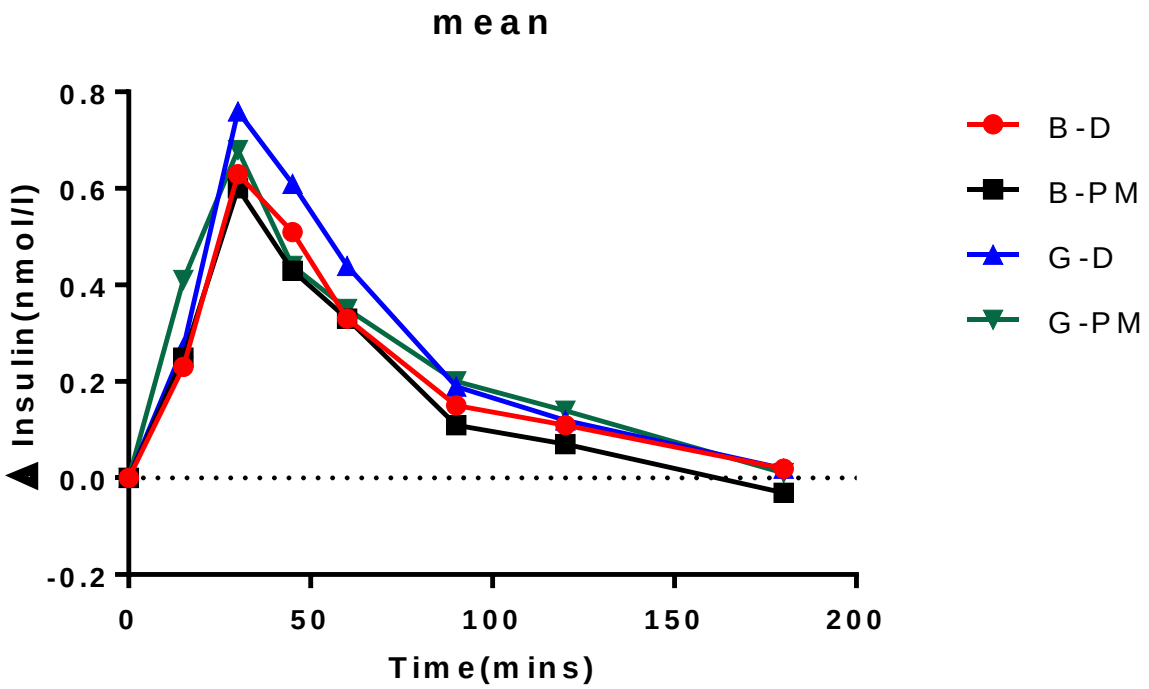

Figure 7-Insulin response following intake of the various test meals. Values are expressed as mean±SEM; n=20

Table 4a-Insulin iAUC and change in percentage when compared to reference (0-15, 0-30 and 0-45) (Untransformed mean values are shown) Values in the same column with different superscript letters are significantly different, P < 0.05 (ANOVA followed by Tukey's multiple comparisons test)

Time	**0-15**		**0-30**		**0-45**	
Meal	iAUC (nmol.min/L)	Change (%)	iAUC (nmol.min/L)	Change (%)	iAUC (nmol.min/L)	Change (%)
B-D(Ref)	1.69±0.28[a]	-	8.11±1.10[a]	-	16.65±2.14[a]	-
B-PM	2.10±0.48[a]	+24.26	8.57±1.60[a]	+5.67	16.25±2.58[a]	-2.40
G-D	2.01±0.42[a]	+18.93	9.69±1.44[b]	+19.48	19.92±2.59[a]	+17.53
G-PM	3.05±0.50[b]	+80.47	11.17±1.46[b]	+37.73	19.57±2.41[a]	+21.65

Table 4b-Insulin iAUC and change in percentage when compared to reference (0-120 and 0-180) (Untransformed mean values are shown)

Time	**0-120**		**0-180**	
Meal	iAUC (nmol.min/L)	Change (%)	iAUC (nmol.min/L)	Change (%)
B-D(Ref)	33.15±4.72[a]	-	38.28±5.33[a]	-
B-PM	32.041±4.84[a]	-3.34	36.10±5.53[a]	-5.69
G-D	42.60±6.02[a]	+28.50	46.03±7.41[a]	+20.24
G-PM	38.17±5.99[a]	+15.14	43.66±7.29[a]	+14.05

Table 5-Change in iPeak values (Values expresses as mean±sem)

Meal	**iPeak value (∆nmol/l)**	**Change (%)**
B-D (Ref)	0.63±.080[a]	-
B-PM	0.60±.084[a]	-5%
G-D	0.76±.095[a]	+20.63
G-PM	0.68±.085[a]	+7.93

A significant difference was found between G-PM and the remaining drinks in the earliest post prandial window (0-15 mins).However in the 0-30 window G-PM was found to significantly differ with the two placebo drinks and not G-D. No other significant differences were found in any of the remaining time intervals.

5.3 Appetite data analysis

The data from the computerized questionnaires on satiety, desire to eat and hunger were analyses using ANCOVA followed by Tukey's comparison test. In the case of data of "desire to eat" and "hunger" it was found that some subjects had not filled in the questionnaire at all time points and so these subjects were removed from the analysis. In the case of the "desire to eat" data only 14 subjects were used in the analysis and in the case of the "hunger" 18 subjects were used. No significant differences were found in any of the data.

6 Discussion

The results showed a statistically significant difference between the insulin iAUC's of the G-PM meal and the remaining drinks in the 0-15 min time interval. In the 15-30 min time interval though, both G-PM and G-D were found to be significantly different from the placebos though not from each other. Hence it would appear that the insulin responses for the G meals corresponds to the expected outcome of obtaining an earlier insulin response. This is particularly apparent in the case of the G-PM meal where by giving half the dose of the active ingredients prior to the meal, the insulin response was able to come very early (i.e. 0-15 min time interval).While no significant differences were found between the glucose iAUC's of all the meals, a significant difference was found between the iPeak values of G-D and G-PM (seen in table 3) which could possibly be caused by the above mentioned increase in early insulin levels in G-PM as compared to G-D.

As mentioned previously, studies using similar versions of the test drinks have been conducted before. In the first study conducted by A. Forslund et al (unpublished) the researchers discovered that there was a significant reduction in post prandial glucose levels following consumption of the meal as compared to the reference drink. More interestingly though they discovered that while the test drink variants had higher early postprandial insulin levels (0-15 mins) as compared to the reference this increase was significantly much higher in the drink containing amino acids alone indicating improved insulin economy caused by CrPic.

In the second study conducted by Svensson et al (unpublished) the test drink was tested more in the form of dose-response manner. The test drink in this case had four variants one a placebo and the remaining containing mixtures of amino acids and CrPic in increasing amounts. The subjects were told to consume part of the drink prior to the meal and the remaining during the meal consisting of mashed potatoes, cod fillet, butter and jam. Much like the above mentioned study the researchers found that there was a relatively similar reduction in postprandial glucose levels in all three variants of the test drink. More interestingly though they discovered that the mixture containing the highest dose of the active components had a much higher increase in early postprandial insulin levels while the other two doses showed no significant increase as compared to the reference that it may be possible

to lower the amino acid dose to get a reduction in glycaemia, without a concomitant increase in insulin.

Since the meal plan used in this study was thought to be identical to the current study and the composition of the test drink used in this study was almost similar to the composition of the second variant of the test drink (3.5 g AA and 250 µg CrPic) used in the study by Svensson et al, the results of the present study were expected to be relatively similar to those obtained in their study. However this was not the case. Unlike the previous two studies no significant reduction in postprandial glycaemia was observed. Instead there appeared to be an unexpected although statistically insignificant increase in overall post prandial glucose levels (see table 2a and 2b).Also the glucose iPeak value for "G-PM" was lower than the reference (see table 3).

The postprandial insulin levels were also found to increase in this study which seems to initially concur with the results of the previous studies. But as mentioned in the paragraph above unlike the previous studies the increase in post prandial insulin levels were not accompanied by simultaneous reduction in postprandial glucose levels and instead the glucose levels were found to increase (particularly in the case of G-D) for some unknown reason. A significant increase in insulin levels in the G-PM meals was observed in the early postprandial window (0-15 and 0-30, see table 4a) and this could possibly account for the previously mentioned reduction in glucose iPeak value. After examining tables 2a, 2b, 4a and 4b it would appear that in the case of the G-D meal, the increase in insulin seems to be to counter the increase in glucose levels. This also appears to be the case in the case of the G-PM (looking at the 0-120 and 0-180 time intervals) meal as well although it would appear that the relatively large increase in early insulin levels (0-15 and 0-30 time intervals, table 4b) may have possibly blunted the corresponding glucose response to a certain extent as well and this could account for the relatively small increase in glucose levels in G-PM as compared to G-D(see table 2a and 2b).It could also possibly account for the significant difference found between the iPeak values of G-PM and G-D.

It would also appear that unlike the study conducted by A. Forslund et al (unpublished), there was no improvement in insulin economy (particularly more so in the case of G-PM) though it must be noted that the dose of CrPic shown to have an insulin sensitizing effect was 500 µg in that particular study while the dose used in the current study was only 250 µg. Also a meta-analysis of nearly 41 studies on Cr supplementation showed no statistically significant effect of Cr on lipid and glucose metabolism in subjects without diabetes though in the case of subjects with diabetes there was significant reduction in glycemic profiles(Balk et al., 2007) indicating that at the very least there are a still a lot of discrepancies in the effects of Cr supplementation in healthy individuals and more research still needs to be conducted in order to deliver a definite outcome

Clearly there appears to be a difference in the results obtained in this study from the previous studies and one possible explanation for that is that just after finishing the study it was realized that the potato mash used in Svensson et al was Ica Basic while the potato mash use in the current study was Ica with the difference being that the latter contained milk powder. The concentration of ingredients between the two mashes were as follows

- ICA basic- 99 % potato
- ICA – 90 % potato and 7 % milk powder

So in order to determine if the milk in the potato mash may have influenced the results a second trial was run consisting of five subjects. The subjects were made to repeat the "B-PM" test again exactly with the exception that the potato mash used this time was ICA Basic. Milk is known to be quiet insulinotropic in nature (Östman, Liljeberg Elmståhl and Björck, 2001). However upon analysis it was found that no significant differences were found in insulin iAUC's and that the mean glucose iAUC for ICA was actually slightly higher than ICA Basic (not of statistical significance though)(results not shown).

There however exists the possibility that since the sample size was quiet small in the secondary trial the results may not necessarily be statistically significant. Also among the five subjects two were considered non responders (based on the classification used in A.Forslund et al.) to the drink while one had an unusual response which in essence further decreases the effective sample size. If this were to be the case it is possible that the milk fraction in the potato mash may have compromised the results. While most studies have shown the insulinotropic nature of milk generally accompanied by a reduction in corresponding blood glucose levels (Östman, Liljeberg Elmståhl and Björck, 2001), one study in particular had results slightly different from the rest (Liljeberg Elmståhl and Björck, 2001). The objective of this particular study was to determine the impact of milk on a high GI meal (white bread) and low GI (spaghetti) meal respectively. The test subjects were made to drink different amounts of milk (200 and 400 ml) with these meals as well as reference. Similar to the current study, the researchers found no significant difference in post prandial glucose levels after ingestion of milk in either of the meals as compared to the reference. However the glucose AUC's (0-95 mins) for the high GI meals had very interesting results. The results showed a very odd pattern in the blood glucose AUC's in response to milk. In the case of the high GI meal, the 200 ml test had glucose AUC's lower than the reference while the 400 ml test had the opposite result. In the low GI meal, the 200 ml test had a glucose AUC higher than the reference while the 400 ml test had an opposite result in this case indicating that the glucose response to milk does not have a linear pattern and can be possibly quite unpredictable. Assuming the milk fraction did compromise the results in the current study it is possible that a similar scenario to the study above may have occurred here as well. Also since the increase in blood glucose levels were found to be statistically insignificant there exists the possibility that the increase could be due to chance fluctuations in the results.

Recent studies have shown that meal order can play an important role in glucose and insulin regulation (Shukla et al, 2015, Trico et al, 2015). Based on the data in table 4a and 4b it would appear that ingesting the test drink prior to meal consumption seems to augment the early postprandial insulin response to glucose as compared to ingesting it during the meal. Besides this in a recent study conducted by Gunnerud et al in 2012, the researchers found that intake of pre-meal drinks containing whey protein and amino acids mixture(the same amino acids used in the current study) or whey protein alone with a standardized composite ham sandwich meal favoured an increase in early post prandial insulin response (0-15 min).However what was even more interesting was that while both the whey protein alone as well as the mixture of whey protein and amino acids seemed to favour an increase in early postprandial insulin levels the insulin iAUC (0-15 mins) for the whey and amino acid mixture was slightly higher than the insulin iAUC (0-15 mins) of the whey protein alone. The insulinotropic nature of milk is theorised to be in part from its whey fraction (Nilsson, J Holst and ME Björck, 2007) so it is plausible that similar to the study conducted by Gunnerud et al, the milk fraction in the potato mash and the amino acids may have had a concerted insulinotropic effect resulting in the statistically insignificant increase in the early post prandial insulin levels (0-15 mins) seen in the case of G-PM.

Both Cr and whey have been suggested to influence appetite (Anton et al., 2008, Veldhorst et al., 2009) and decrease body weight gain (Martin et al., 2006, Veldhorst et al., 2009) respectively. It has also been shown that insulin can regulate appetite and suppress food intake (Stanley et al., 2005). However no significant differences were found in the appetite questionnaires in this study.

7 Conclusion

Based on the discussion it would appear that ingesting the test drink prior to the meal seems to favor an increase in early postprandial insulin levels (0-15 mins) as compared to ingesting it during the meal.

That being said it would appear that the results have some unexplained discrepancies (the unexpected increase in glucose levels) and hence may not be conclusive enough to elicit a definite outcome and instead it would probably be much wiser to repeat the study with the original sample size using the ICA basic mash.

8 References

- Anton, S., Morrison, C., Cefalu, W., Martin, C., Coulon, S., Geiselman, P., Han, H., White, C. and Williamson, D. (2008). Effects of Chromium Picolinate on Food Intake and Satiety. *Diabetes Technology & Therapeutics*, 10(5), pp.405-412.
- Augustin, L., Kendall, C., Jenkins, D., Willett, W., Astrup, A., Barclay, A., Björck, I., Brand-Miller, J., Brighenti, F., Buyken, A., Ceriello, A., La Vecchia, C., Livesey, G., Liu, S., Riccardi, G., Rizkalla, S., Sievenpiper, J., Trichopoulou, A., Wolever, T., Baer-Sinnott, S. and Poli, A. (2015). Glycemic index, glycemic load and glycemic response: An International Scientific Consensus Summit from the International Carbohydrate Quality Consortium (ICQC). *Nutrition, Metabolism and Cardiovascular Diseases*, 25(9), pp.795-815.
- Balk, E., Tatsioni, A., Lichtenstein, A., Lau, J. and Pittas, A. (2007). Effect of Chromium Supplementation on Glucose Metabolism and Lipids: A systematic review of randomized controlled trials.*Diabetes Care*, 30(8), pp.2154-2163.
- Bhattacharya, S., Dey, D. and Roy, S. (2007). Molecular mechanism of insulin resistance.*Journal of Biosciences*, 32(2), pp.405-413.
- Brown, R., Forloines-Lynn, S., Cross, R. and Heizer, W. (1986). Chromium deficiency after long-term total parenteral nutrition. *Digest Dis Sci*, 31(6), pp.661-664.
- Codario, R. (2011). *Type 2 diabetes, pre-diabetes, and the metabolic syndrome*. Totowa, N.J.: Humana Press.
- Davidenko, O., Darcel, N., Fromentin, G. and Tomé, D. (2013). Control of protein and energy intake - brain mechanisms. *European Journal of Clinical Nutrition*, 67(5), pp.455-461.
- DeFronzo, R. and Ferrannini, E. (1991). Insulin Resistance: A Multifaceted Syndrome Responsible for NIDDM, Obesity, Hypertension, Dyslipidemia, and Atherosclerotic Cardiovascular Disease.*Diabetes Care*, 14(3), pp.173-194.
- de Luca, C. and Olefsky, J. (2007). Inflammation and insulin resistance. *FEBS Letters*, 582(1), pp.97-105.
- Flint, A., Gregersen, N., Gluud, L., Møller, B., Raben, A., Tetens, I., Verdich, C. and Astrup, A. (2007). Associations between postprandial insulin and blood glucose responses, appetite sensations and energy intake in normal weight and overweight individuals: a meta-analysis of test meal studies. *BJN*, 98(01), p.17.
- Flint, A., Raben, A., Blundell, J. and Astrup, A. (2000). Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. *International Journal of Obesity*, 24(1), pp.38-48.
- Forslund, A., Öste, R., Björck, I. and Östman, E. (to be submitted). A drink containing a mix of amino acids and chromium picolinate improves postprandial glycaemia at breakfast in healthy, overweight subjects.
- Evans, G. and Bowman, T. (1992). Chromium picolinate increases membrane fluidity and rate of insulin internalization. *Journal of Inorganic Biochemistry*, 46(4), pp.243-250.
- Evans, G. and Pouchnik, D. (1993). Composition and biological activity of chromium-pyridine carboxylate complexes. *Journal of Inorganic Biochemistry*, 49(3), pp.177-187.
- Floyd, J., Fajans, S., Conn, J., Knopf, R. and Rull, J. (1966). Stimulation of insulin secretion by amino acids. *Journal of Clinical Investigation*, 45(9), pp.1487-1502.

- Frid, A., Nilsson, M., Holst, J. and Björck, I. (2005). Effect of whey on blood glucose and insulin responses to composite breakfast and lunch meals in type 2 diabetic subjects. *American Journal of clinical Nutrition*, 82, pp.69-75.
- Gannon, M., Nuttall, F., Lane, J. and Burmeister, L. (1992). Metabolic response to cottage cheese or egg white protein, with or without glucose, in type II diabetic subjects. *Metabolism*, 41(10), pp.1137-1145.
- Gunnerud, U., Heinzle, C., Holst, J., Östman, E. and Björck, I. (2012). Effects of Pre-Meal Drinks with Protein and Amino Acids on Glycemic and Metabolic Responses at a Subsequent Composite Meal. *PLoS ONE*, 7(9), p.e44731.
- Han, T. and Lean, M. (2011). Metabolic Syndrome. *Medicine*, 39(1), pp.24-31.
- Hepburn, D. and Vincent, J. (2002). In Vivo Distribution of Chromium from Chromium Picolinate in Rats and Implications for the Safety of the Dietary Supplement. *Chem. Res. Toxicol.*, 15(2), pp.93-100.
- Holst, J. and Ørskov, C. (2001). Incretin hormones - an update. *Scandinavian Journal of Clinical and Laboratory Investigation*, 61(7), pp.75-85.
- Huang, S., Peng, W., Jiang, X., Shao, K., Xia, L., Tang, Y. and Qiu, J. (2014). The Effect of Chromium Picolinate Supplementation on the Pancreas and Macroangiopathy in Type II Diabetes Mellitus Rats. *Journal of Diabetes Research*, 2014, pp.1-8.
- Hussain, A., Claussen, B., Ramachandran, A. and Williams, R. (2006). Prevention of type 2 diabetes: A review. *Diabetes Research and Clinical Practice*, 76(3), pp.317–326.
- Ignell, C 2015, *Gestational Diabetes Mellitus. [Elektronisk Resurs] : Prevalence In Southern Sweden And Risk Factors For Subsequent Diabetes*, n.p.: Malmö : Department of Clinical Sciences, Malmö, Lund University, 2015, Library catalogue (Lovisa), EBSCO*host*, viewed 3 October 2016.
- International Diabetes Federation, (2015). *IDF Diabetes Atlas*. 7th ed. Brussels,Belgium: International Diabetes Federation.
- International Diabetes Federation. (2016). *IDF Worldwide Definition of the Metabolic Syndrome*. [online] Available at: http://www.idf.org/metabolic-syndrome [Accessed 6 Sep. 2016].
- International Diabetes Federation. (2016). *Diabetes: facts and figures*. [online] Available at: http://www.idf.org/about-diabetes/facts-figures [Accessed 6 Sep. 2016].
- Kapur, S., Groves, M., Zava, D. and Kapur, S. (2010). Postprandial Insulin and Triglycerides after Different Breakfast Meal Challenges: Use of Finger Stick Capillary Dried Blood Spots to Study Postprandial Dysmetabolism. *Journal of Diabetes Science and Technology*, 4(2), pp.236-243.
- Keane, K. and Newsholme, P. (2014). Metabolic Regulation of Insulin Secretion. *Vitamins and Hormones*, 95, pp.16-19.
- Liljeberg Elmståhl, H. and Björck, I. (2001). Milk as a supplement to mixed meals may elevate postprandial insulinaemia. *European Journal of Clinical Nutrition*, 55(11), pp.994-999.
- Lindgren, O., Pacini, G., Tura, A., Holst, J., Deacon, C. and Ahrén, B. (2015). Incretin Effect After Oral Amino Acid Ingestion in Humans. *The Journal of Clinical Endocrinology & Metabolism*, 100(3), pp.1172-1176.

- Lionetti, L., Mollica, M., Lombardi, A., Cavaliere, G., Gifuni, G. and Barletta, A. (2009). From chronic overnutrition to insulin resistance: The role of fat-storing capacity and inflammation. *Nutrition, Metabolism and Cardiovascular Diseases*, 19(2), pp.146-152.
- Martin, J., Wang, Z., Zhang, X., Wachtel, D., Volaufova, J., Matthews, D. and Cefalu, W. (2006). Chromium Picolinate Supplementation Attenuates Body Weight Gain and Increases Insulin Sensitivity in Subjects With Type 2 Diabetes. *Diabetes Care*, 29(8), pp.1826-1832.
- National Institute of Diabetes and Digestive and Kidney Diseases. (2016). *Types of Diabetes | NIDDK*. [online] Available at: https://www.niddk.nih.gov/health-information/diabetes/types [Accessed 6 Sep. 2016].
- Nilsson, M., J Holst, J. and ME Björck, I. (2007). Metabolic effects of amino acid mixtures and whey protein in healthy subjects: studies using glucose-equivalent drinks. *American Journal of Clinical Nutrition*, 85, pp.996-1004.
- Nolan, J. (2006). What is type 2 diabetes?. *Medicine*, 34(2), pp.52-56.
- Östman, E., Liljeberg Elmståhl, H. and Björck, I. (2001). Inconsistency between glycemic and insulinemic responses to regular and fermented milk products. *American Journal of Clinical Nutrition*, (74), pp.96-100.
- Paiva, A., Lima, J., Medeiros, A., Figueiredo, H., Andrade, R., Ururahy, M., Rezende, A., Brandão-Neto, J. and Almeida, M. (2015). Beneficial effects of oral chromium picolinate supplementation on glycemic control in patients with type 2 diabetes: A randomized clinical study. *Journal of Trace Elements in Medicine and Biology*, 32, pp.66-72.
- Parsaeyan, N. and Mozaffari–Khosravi, H. (2012). Effect of Chromium Supplementation on Blood Glucose, Hemoglobin A1c, Lipid Profile and Lipid Peroxidation in Type 2 Diabetic Patients. *Iranian Journal of Diabetes and Obesity*, 4(4).
- Pattar, G., Tackett, L., Liu, P. and Elmendorf, J. (2006). Chromium picolinate positively influences the glucose transporter system via affecting cholesterol homeostasis in adipocytes cultured under hyperglycemic diabetic conditions. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, 610(1-2), pp.93-100.
- Prasanna Kumar, K. (2011). Metabolic Syndrome. *Intentional Journal of Diabetes in Developing Countries*, 31(4), pp.185-187.
- Rosén, L., Silva, L., Andersson, U., Holm, C., Östman, E. and Björck, I. (2009). Endosperm and whole grain rye breads are characterized by low post-prandial insulin response and a beneficial blood glucose profile. *Nutrition Journal*, 8(1).
- Saeed, A., Jones, S., Nuttall, F. and Gannon, M. (2002). A fasting-induced decrease in plasma glucose concentration does not affect the insulin response to ingested protein in people with type 2 diabetes. *Metabolism*, 51(8), pp.1027-1033.
- Salehi, A., Gunnerud, U., Muhammed, S., Östman, E., Holst, J., Björck, I. and Rorsman, P. (2012). The insulinogenic effect of whey protein is partially mediated by a direct effect of amino acids and GIP on β-cells. *Nutrition & Metabolism*, 9(1), p.48.
- Samocha-Bonet, D., Chisholm, D., Holst, J. and Greenfield, J. (2015). L-Glutamine and Whole Protein Restore First-Phase Insulin Response and Increase Glucagon-Like Peptide-1 in Type 2 Diabetes Patients. *Nutrients*, 7(4), pp.2101-2108.

- Scientific Opinion on the safety of trivalent chromium as a nutrient added for nutritional purposes to foodstuffs for particular nutritional uses and foods intended for the general population (including food supplements). (2010). *EFSA Journal*, 8(12), p.1882.
- Shinde, U., Sharma, G., Xu, Y., Dhalla, N. and Goyal, R. (2004). Insulin sensitising action of chromium picolinate in various experimental models of diabetes mellitus. *Journal of Trace Elements in Medicine and Biology*, 18(1), pp.23-32.
- Shukla, A., Iliescu, R., Thomas, C. and Aronne, L. (2015). Food Order Has a Significant Impact on Postprandial Glucose and Insulin Levels. *Diabetes Care*, 38(7), pp.e98-e99.
- Simpson, R., Shaw, J. and Zimmet, P. (2003). The prevention of type 2 diabetes — lifestyle change or pharmacotherapy? A challenge for the 21st century. *Diabetes Research and Clinical Practice*, 59(3), pp. Taylor, J., Kraja, A., de las Fuentes, L., Stanfill, A., Clark, A. and Cashion, A. (2013). An Overview of the Genomics of Metabolic Syndrome. *Journal of Nursing Scholarship*, 45(1), pp.52-59.165-180.
- Stanley, S., Wynne, K., McGowan, B. and Bloom, S. (2005). Hormonal Regulation of Food Intake. *Physiological Reviews*, 85(4), pp.1131-1158.
- Suksomboon, N., Poolsup, N. and Yuwanakorn, A. (2014). Systematic review and meta-analysis of the efficacy and safety of chromium supplementation in diabetes. *J Clin Pharm Ther*, 39(3), pp.292-306.
- Svensson, A., Öste, R., Björck, I. and Östman, E. (to be submitted). Enclosure of low doses of amino acids and chromium picolinate in table water lowers glycaemia to a composite lunch-like meal in healthy overweight subjects.
- Taylor, J., Kraja, A., de las Fuentes, L., Stanfill, A., Clark, A. and Cashion, A. (2013). An Overview of the Genomics of Metabolic Syndrome. *Journal of Nursing Scholarship*, 45(1), pp.52-59.
- Torres, N., Noriega, L. and Tovar, A. (2016). Nutrient Modulation of Insulin Secretion.*Vitamins and Hormones*, 80, pp.217-244.
- Tricò, D., Baldi, S., Tulipani, A., Frascerra, S., Macedo, M., Mari, A., Ferrannini, E. and Natali, A. (2015). Mechanisms through which a small protein and lipid preload improves glucose tolerance.*Diabetologia*, 58(11), pp.2503-2512.
- Veldhorst, M., Nieuwenhuizen, A., Hochstenbach-Waelen, A., van Vught, A., Westerterp, K., Engelen, M., Brummer, R., Deutz, N. and Westerterp-Plantenga, M. (2009). Dose-dependent satiating effect of whey relative to casein or soy. *Physiology & Behavior*, 96(4-5), pp.675-682.
- Veldhorst, M., Smeets, A., Soenen, S., Hochstenbach-Waelen, A., Hursel, R., Diepvens, K., Lejeune, M., Luscombe-Marsh, N. and Westerterp-Plantenga, M. (2008). Protein-induced satiety: Effects and mechanisms of different proteins. *Physiology & Behavior*, 94(2), pp.300-307.
- Wang, Y. and Yao, M. (2009). Effects of chromium picolinate on glucose uptake in insulin-resistant 3T3-L1 adipocytes involve activation of p38 MAPK. *The Journal of Nutritional Biochemistry*, 20(12), pp.982-991.
- World Health Organization. (2016). *Diabetes*. [online] Available at: http://www.who.int/mediacentre/factsheets/fs312/en/ [Accessed 6 Sep. 2016].
- Widmaier, E., Raff, H. and Strang, K. (2014). *Vander's Human Physiology-The mechanisms of body function*. 13th ed. New York: McGraw-Hill.

- Zhang, P., Zhang, X., Brown, J., Vistisen, D., Sicree, R., Shaw, J. and Nichols, G. (2010). Global healthcare expenditure on diabetes for 2010 and 2030. *Diabetes Research and Clinical Practice*, 87(3), pp.293-301.