GASTRIC EMPTYING IN HUMANS; CARBOHYDRATE INGESTION, GASTROINTESTINAL HORMONES AND GENETIC VARIATION

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"Now this is not the end. It is not even the beginning of the end. But it is, perhaps the end of the beginning." [Winston Churchill]

Abstract

The prevalence of overweight and obesity continues to rise substantially across the world. It is the leading preventable cause of death worldwide and is associated with a large number of comorbidities that present a perpetual burden on healthcare costs. Much of the recent work to understand and address the problem of obesity has focused on the role of gastrointestinal hormones on the regulation of appetite, satiety, and food intake, and how interventions such as physical activity and exercise can affect the secretion of these hormones. However, the gastrointestinal system and the role of gastric emptying are often overlooked. The aim of this thesis was to enhance understanding of the physiology and regulation of gastric emptying and its interactions with carbohydrates. This will help in the development of novel non-pharmacological dietary interventions or foods that can modulate appetite and energy intake.

A series of studies on human volunteers are presented in this thesis. Firstly, the gastric emptying rate of different 6% simple sugar solutions (water control, fructose, glucose, sucrose, 50:50 fructose and glucose) and gut hormone responses of circulating acylated ghrelin, active glucagon like peptide-1 (GLP-1), glucose dependent insulinotropic polypeptide (GIP) and insulin were investigated. Hepatic metabolism and function in response to the different simple sugar solutions were also examined. The time of maximal gastric emptying rate (T_{lag}) differed significantly between between sucrose and glucose solutions. Differences in insulin and GIP responses between fructose containing solutions and glucose only solutions were also seen. No differences in hepatic metabolism measures or function were observed following the intake of 36 g of the various test sugars. However, lactate production was significantly greater for fructose containing solutions. Following on from these results, the effect of increased dietary fructose intake on gastric emptying rate of glucose and fructose was investigated. Three days supplementation with 120g/d fructose resulted in acceleration of gastric emptying rate of a fructose but not a glucose solution. No significant differences in the circulating concentration of gastrointestinal hormones, but subtle differences in responses over time were suggested which may explain the specific monosaccharide adaptations of gastric emptying. Further work is required to confirm this and to investigate the longevity and reversibility of the gastrointestinal adaptation and the mechanism involved. Lastly, several tagging single nucleotide polymorphisms (SNP) of the GLP-1 receptor gene were associated with gastric emptying rate. Further work is required on the regions identified to pinpoint the exact SNP or SNPs responsible.

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Publications

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List of Abbreviations and Symbols

°	Degree
°C	Degree celcius
µl	Microlitre
µM	Micromole
$^{12}CO_2$	Carbon 12 carbon dioxide
$^{13}CO_2$	Carbon 13 carbon dioxide
3D	Three dimension
^{99}mTc-SC	99mTechnetium-sulphur colloid
acetyl-coA	Acetyle coenzyme A
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
AUC	Area under curve
BM	Body mass
BMI	Body mass index
^{13}C	Carbon 13
C_{max}	Peak blood concentration
CCK	Cholecystokinin
cm	Centimetre
CNS	Central nervous system
d	Day
DOB	Delta over baseline
DPP-IV	Dipeptidyl peptidase IV
et al.	*et alii*
FC	Fructose control (trial)
FRU	Fructose (trial)
FS	Fructose with supplementation (trial)
g	Gram
g	Gravitational acceleration
GC	Glucose control (trial)
Gd-DOTA	Gadolinium tetraazacyclododecane tetraacetic acid
GHS-R	Growth hormone secretagogue receptor
GI	Gastrointestinal
GIP	Glucose dependent insulinotropic polypeptide
GLP-1	Glucagon like peptide-1
GLP-1R	Glucagon like peptide-1 receptor
GLU	Glucose (trial)
GLU+FRU	Glucose and fructose (trial)
GLUT2	Glucose transporter 2
GLUT5	Glucose transporter 5
GNAS1	Guanine nucleotide binding protein G alpha subunit 1
GOD-PAP	Glucose oxidase phenol 4-aminoantipyrine peroxidase
GS	Glucose with supplementation (trial)

^{2}H	Hydrogen isotope deuterium
$^{2}H_2O$	Deuterium oxide
h	Hour
HFCS	High fructose corn syrup
IR	Infrared
kg	Kilogram
Km	Michaelis constant
L	Litre
LEPR	Leptin receptor
Ltd	Limited
m	Metre
MC4R	Alpha melanocortin 4 receptor
mCi	Millicurie
mg	Milligram
min	Minute
mL	Millilitre
mm	Millimetre
mmol	Millimoles
mOsmol	Milliosmoles
Mrad	Millirad
MRI	Magnetic resonance imaging
n	Participant number
NAFLD	Non-alcoholic fatty liver disease
NEFA	Non esterified fatty acid
ng	Nanogram
NPY2R	Neuropeptide Y2 receptor
OXM	Oxyntomodulin
P	Probability
pg	Picogram
POMC	Pro-opiomelanocortin
PP	Pancreatic polypeptide
PYY	Peptide tyrosin tyrosin
r	Pearson product moment correlation coefficient
Ranbut	D-3 Hydroxybutyrate assay
SD	Standard deviation
sec	Second
SGLT1	Sodium dependent glucose transporter 1
SNP	Single nucleotide polymorphism
SUC	Sucrose (trial)
$T_{½}$	Half emptying time
T_{lag}	Time of maximal emptying rate
T_{max}	Time to reach peak blood concentration
U	Unit
UK	United Kingdom

USA	United States of America
VAS	Visual analogue scale
W	Water (trial)
WHO	World Health Organisation
y	Year

1. GENERAL INTRODUCTION AND BACKGROUND

1.1. OBESITY

Overweight and obesity are defined by the World Health Organisation (WHO) as abnormal or excessive fat accumulation that may impair health (WHO, 2014). Classification of being overweight is commonly described as having a body mass index (BMI) $\geq$ 25 kg/m^2 while that of obesity is $\geq$ 30 kg/m^2. In 2008, an estimated world-wide population of 1.4 billion (35%) adults were classed as overweight, of which 500 million (11%) were deemed to be obese (WHO, 2014). Furthermore, more than 40 million children under the age of 5 were considered to be overweight or obese in 2012 (WHO, 2014). Obesity has, therefore, been an important topic and a concern in the health care science over the last few decades. It remains a growing epidemic in most countries around the world with global predictions of 3.3 billion adults being overweight of which 1.12 billion will be obese by 2030 (Kelly, Yang, Chen, Reynolds & He, 2008). Obesity is the leading preventable cause of death worldwide, contributing extensively to comorbidities such as heart disease, type II diabetes, certain types of cancer and other disorders (Mushref & Srinivasan, 2013). In its broadest sense, obesity can be considered the result of an imbalance of energy intake and energy expenditure where intake exceeds that of expenditure (Little, Horowitz & Feinle-Bisset, 2007). In substance the factors influencing food or energy intake and energy expenditure are multifactorial and complex with interaction between dietary environment, genetic, metabolic, behavioural and physiological factors all being implicated (Cheung & Mao, 2012; Castiglione, Read & French, 2002).

To understand and tackle the rising problem of obesity, much research has been conducted in recent times on subjective feelings of appetite, food intake, and their effect on energy balance. The predominant focus of research in this area has been on the regulation of appetite and satiety by gut-derived hormones, and how interventions such as physical activity and exercise may affect these. Physical activity and exercise along with dietary restriction are often prescribed as effective non-surgical treatments and prevention strategies for reducing or controlling overweight and obesity. Pharmacological treatments are also utilised in the treatment of obesity but the side effects that can result from the use of existing pharmacological agents are far from desirable (Xia & Grant, 2013). For example, sibutramine use causes strain on the cardiovascular system through increased heart rate and blood pressure (Berkowitz, Wadden, Tershakovec & Cronquist, 2003) and orlistat is associated with gastrointestinal side effects and requires supplementation and monitoring of vitamin

levels (McDuffie, Calis, Booth, Uwaifo & Yanovski, 2002). Furthermore, in extreme cases where bariatric surgery is utilised to promote weight loss, deficiencies in macro- and micronutrients are common and drug absorption is compromised (Stein, Stier, Raab & Weiner, 2014). Therefore, there is still a need for the identification of better therapeutic intervention targets to prevent morbid fat accumulation in the normal population (Xia & Grant, 2013). In research for both the prevention and treatment of obesity, the gastrointestinal system and the role of gastric emptying in appetite and satiety are often overlooked. Gastric emptying is the process whereby food or foodstuffs are emptied from the stomach into the small intestine where it can then be absorbed. As such, gastric emptying rate is a limiting step in the absorption of nutrients in the gastrointestinal tract, and it seems pivotal that greater consideration should be given to its role in obesity. Greater understanding of the physiology and regulation of gastric emptying and its interactions with nutrients may lead to the development of new non-pharmacological dietary interventions or foods that can affect appetite and thereby modulate energy intake.

1.2. ANATOMY AND FUNCTION OF THE GASTROINTESTINAL SYSTEM

The gastrointestinal system is extensively connected anatomically and physiologically to the nervous, cardiovascular, endocrine and lymphoid systems. It consists of the gastrointestinal tract; which includes the mouth, pharynx, oesophagus, stomach, duodenum, small intestine, large intestine, and rectum, and incorporates a large heterogeneous collection of disparate organs including the salivary glands, liver, pancreas, and gallbladder (Vander Sherman & Luciano, 1994). The gastrointestinal tract acts as an interface between the external and internal milieu of the body (Sernka & Jacobson, 1983) and provides a natural modality for the supply of both the fuel and organic molecules essential for cellular life, function, growth and repair. This fundamental function of the gastrointestinal system, where macro- and micronutrients are extracted from the external environment in the form of food and drink, is achieved through four main processes; digestion, secretion, absorption and motility (Vander *et al*., 1994). Since the majority of digestion and virtually all absorption of nutrients occur in the small intestine, the entrance of ingested food and drink into the small intestine is an important consideration. The stomach, located between the oesophagus and the small intestine, thus plays an important regulatory role in this.

1.2.1. The stomach

The stomach is a J-shaped sack like organ, which is divided into four anatomical regions based on its structure and function; the cardia, fundus, corpus, and pylorus (Martini & Nath, 2009) (Figure 1). The cardia is the smallest region of the stomach sited within 3 cm beyond the lower oesophageal sphincter. Mucous glands are abundant in this region, the alkaline secretions of which coat the junction between the stomach and the oesophagus, protecting the latter from the gastric acid and enzymes. The fundus is the upper section of the stomach superior to the gastro-oesophageal opening. The corpus is the largest region of the stomach spanning between the fundus and the base of the 'J' curve. Gastric glands in the fundus and this central region contain parietal cells, which secrete hydrochloric acid and intrinsic factor, as well as chief cells, which secrete pepsinogen (McPhee & Hammar, 2010). Lastly, the most distal region of the stomach, the pylorus, is divided into the pyloric antrum and pyloric canal and forms the sharp curve of the 'J' shape. The hormone gastrin is secreted in this area from G-cells (Pocock, Richards & Richards, 2013). Mucus and bicarbonate are also secreted by mucous and epithelial cells throughout all regions combining with phospholipids and water to form a protective mucous gel layer for the epithelium (Pocock *et al*., 2013). A muscular sphincter known as the pyloric sphincter separates the stomach and the duodenum.

The stomach has five major functions. 1) It acts as a temporary reservoir for ingested food and drink 2) It performs partial breakdown of food through mechanical action. 3) It permits partial breakdown of food by the chemical action of acid and enzymes. 4) It controls the rate at which food in the form of fluid chyme is delivered to the small intestine for further digestion and subsequent absorption. 5) It produces intrinsic factor, a glycoprotein required for the absorption of vitamin B12 in the small intestine (Martini & Nath, 2009; Hellstrom, Gryback & Jacobsson, 2006; Vander *et al*., 1994).

The wall of the stomach, like the rest of the digestive tract, consists of four distinct layers. The innermost, secondary, tertiary, and outermost layers being the mucosa, the submucosa, the muscularis externa and the serosa, respectively (Martini & Nath, 2009; Vander *et al*., 1994). The mucosa is a mucous membrane comprising of a simple columnar epithelium and a lamina propria composed of areolar tissue. Blood vessels, sensory nerve endings, lymphatic vessels, lymphoid tissue, secretory cells and a thin layer of smooth muscle fibres known as the muscularis mucosae are found in this lamina propria. The smooth muscle fibres in the muscularis mucosae of the stomach are orientated generally in three concentric

layers; the familiar inner circular and outer longitudinal layers found along the digestive tract plus an additional outer layer of circular muscle cells. Furthermore, the internal surface of the stomach in the fundus and body exhibit shallow depressions called gastric pits which lead to gastric glands that extend deep into this lamina propria. The submucosa, a layer of dense connective tissue, contains both large blood vessels and lymphatic vessels and is interlaced with sensory neurons, parasympathetic ganglionic neurons and sympathetic postganglionic fibres that form the submucosal (Meissner's) plexus of the enteric nervous system. The muscularis externa is the primary muscular region of the stomach and is composed again of three layers of muscle; an inner oblique layer, a middle circular layer and an outer longitudinal layer. Sandwiched between the two latter layers lie a second network of sensory neurons, parasympathetic ganglionic neurons, interneurons and sympathetic postganglionic fibres, forming the myenteric (Auerbach's) plexus of the enteric nervous system. Covering the muscularis externa is a serous membrane, the serosa.

When the stomach is empty, the internal mucosa is folded in a series of vertical creases known as rugae, decreasing its volume to approximately 50 mL. These rugae flatten out as the stomach fills, allowing the volume of the stomach to increase. As a result, and due to plasticity of the smooth muscle fibres and activation of a vagal reflex causing inhibition of corpus and fundus smooth muscle tone (relaxation), the storage capacity of the stomach can increase to as much as 1.5 L with little change in intragastric pressure (Varon & Zuleta, 2010; Martini & Nath, 2009; Vander *et al*., 1994; Sanford, 1992) and without causing much discomfort (la Roca-Chiapas & Cordova-Fraga, 2011).

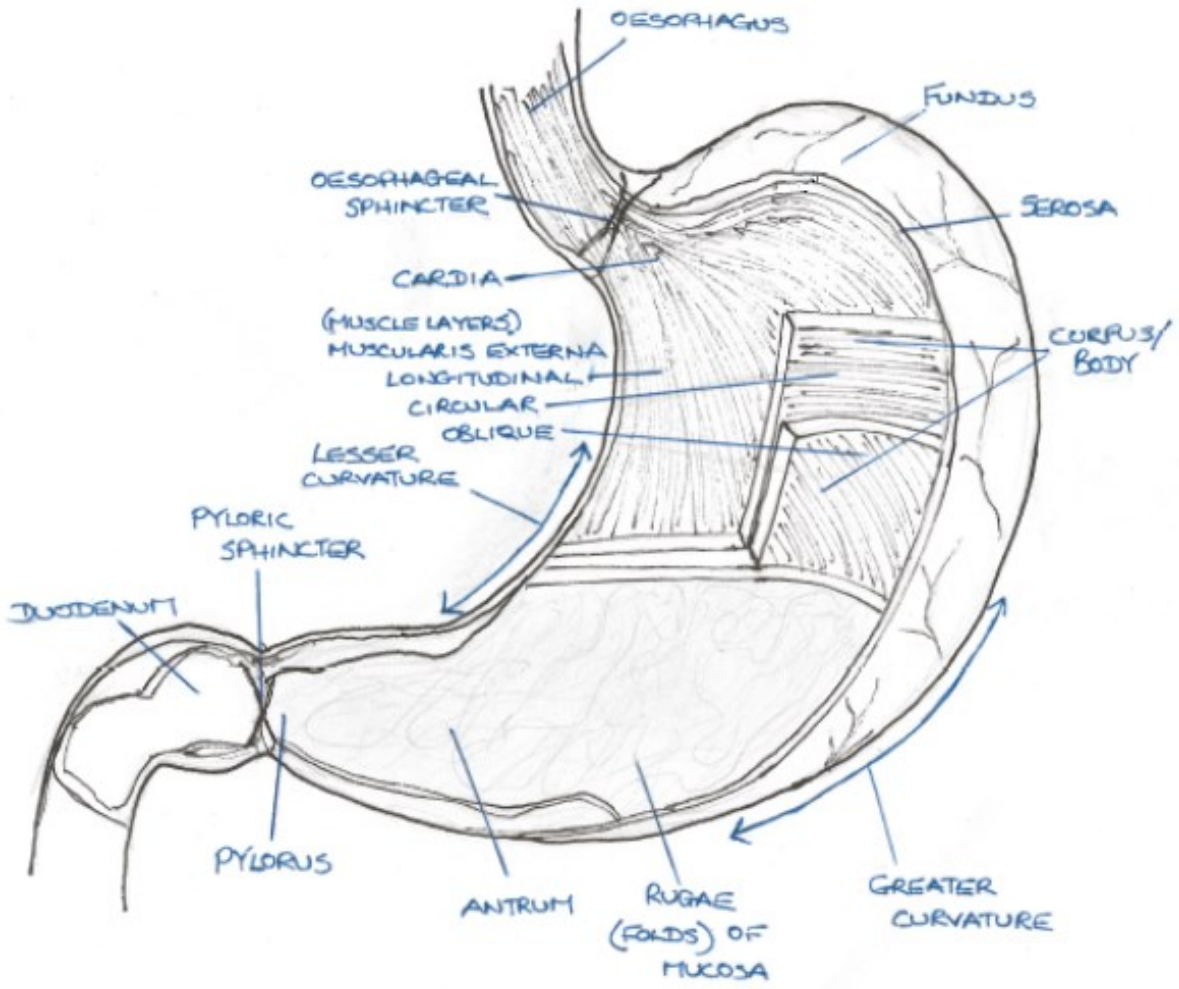

Figure 1. Anatomic regions and structure of the stomach.

1.3. GASTRIC EMPTYING

A basic electrical rhythm exists in the stomach whereby peristaltic waves are generated by pacemaker cells located in the upper great curvature undergoing spontaneous depolarisation-repolarisation cycles at a rate of three per minute (Hellstrom *et al*., 2006; Vander *et al*., 1994). These peristaltic waves propagate distally along the longitudinal smooth muscle of the body, antrum and pylorus (Davenport, 1982), but in the absence of any neural or humoral stimulation, are too weak to initiate an action potential (Vander *et al*., 1994). Upon entry of a meal into the stomach, distension of the wall triggers short and long reflexes through the intrinsic neurons of the myenteric plexus and vagal afferent neurons of the central nervous system, respectively, and acetylcholine and other neurocrine signals are released from the efferent pre- and postganglionic fibres (Sernka & Jacobson, 1983). This increases spike burst

activity, generating action potentials at the peaks of the wave cycle and causing contractions of the muscle layers. The arrival of a peristaltic wave causes the pyloric sphincter to close, constricting the lumen, such that with each muscular contraction of the antrum which forces the stomach's contents towards the pylorus, only a small amount of digested chyme is released into the duodenum. The majority of contents are propelled backwards toward the body contributing to both mixing of contents with gastric juice and mechanical breakdown of food (Kelly, 1980). As the frequency of contraction is determined by the basic electrical rhythm, which remains relatively constant, the rate at which gastric contents (chyme) are emptied from the stomach is dependent on the tone of the pyloric sphincter (Sernka & Jacobson, 1983) and the force of contractions of the antral smooth muscle. This variation in the resistance of flow across the pylorus is influenced by neural and hormonal reflexes and signals (Vander *et al*., 1994; Sernka & Jacobson, 1983). Important distinctions, therefore, are also consequently observed for the emptying of liquids and solids.

1.3.1. Liquids

Ingested liquids distribute rapidly throughout the entire stomach (Hellstrom *et al*., 2006) and are generally emptied from the stomach in an exponential manner with an initial rapid emptying phase followed by a slower linear phase (Vist & Maughan, 1994; Rehrer, Beckers, Brouns, Tenhoor & Saris, 1989; Siegel, Urbain, Adler, Charkes, Maurer, Krevsky *et al*., 1988; Hunt & Spurrell, 1951). This is the result of a low resistance to flow across the pylorus and the rate of emptying is largely determined by the pressure gradient between the stomach and the duodenum (Hellstrom *et al*., 2006; Kelly, 1980). Increases in intragastric pressure have been linearly related to increases in liquid emptying rate (Strunz & Grossman, 1978). As the slow sustained basal electrical rhythm contractions of the proximal stomach play a major role in the regulation of intragastric pressure, and therefore the gastro-duodenal gradient, the proximal stomach is suggested to have a predominant role in the rate of emptying of liquids (Kelly, 1980).

1.3.2. Solids

In contrast to the emptying of liquids, solids empty with an initial slow phase, known as the lag phase, followed by a more rapid linear phase (Siegel *et al*., 1988). This is due to a high resistance to flow across the pylorus that is determined by contractions of the distal antrum

and pylorus (Kelly, 1980). During the lag phase, the ingested solids are redistributed from the fundus and digested into particles of approximately 1-2 mm in size in order for passage through the pylorus (Hellstrom *et al*., 2006; Meyer, Mandiola, Shadchehr & Cohen, 1977). The rapid linear phase of the emptying of solids is similar to the emptying rate of liquids (Siegel *et al*., 1988), as the small digested particles become suspended in the liquid phase of the gastric content and thus empty from the stomach simultaneously (Kelly, 1980). As contractions of the antrum and pylorus determine the resistance of flow of solids and aid the digestion of solid food particles to reduce such resistance, the distal stomach is suggested to play a predominant role in the regulation of the emptying of solids (Kelly, 1980).

1.4. MEASUREMENT OF GASTRIC EMPTYING

A number of different methods are used for the assessment of gastric emptying in clinical and research settings; each having their own advantages and disadvantages. Standard terms and values used for the description and comparison of gastric emptying characteristics include total emptying time, "$T_{½}$" which is the time taken for half of the meal volume to empty and "T_{lag}" which is the time at which the lag phase ends and emptying begins (Hunt & Spurrell, 1951).

Scintigraphy, first described in 1966 by Griffith, Owen, Kirkman & Shields is considered as the 'gold standard' method in determining gastric emptying characteristics (Hellstrom *et al*., 2006). This method involves ingestion of a meal labelled with a radioisotope and repeated anterior and posterior imaging of the gastric area with an external gamma camera (Szarka & Camilleri, 2009; Beckers, Leiper & Davidson, 1992). Regions of interest are identified on all captured images for quantification of radioactivity and counts corrected for depth or distance from the camera, intragastric radioactivity and radioisotope decay (Szarka & Camilleri, 2009; Beckers *et al*., 1992). The advantage of scintigraphy is that both liquid and solid emptying can be assessed with the use of different radioisotope labels. The choice of radiolabel for the emptying of a solid meal requires careful consideration, however, as it needs to remain bound to the solid portion in the stomach, resisting dissociation and emptying with the faster liquid phase of the meal (Szarka & Camilleri, 2009). The first validated solid test meal used consisted of chicken liver labelled with ^{99m}Tc-sulphur colloid (^{99m}Tc-SC). Binding of ^{99m}Tc-SC intracellularly to Kupffer cells involved injecting the radioisotope into a live chicken, sacrificing the animal, then removing and cooking the liver.

More convenient and widely used solid meals at present consist of whole eggs mixed with ^{99m}Tc-SC before cooking which results in fixing of the label to the egg white where it is bound to protein (Szarka & Camilleri, 2009). An obvious disadvantage of scintigraphy is that of radiation exposure. The radiation dose absorbed from exposure to the standardised amount of 0.5 mCi ^{99m}Tc-SC in eggs is 10 mrad (Siegel, Knight, Zelac, Stern & Malmud, 1983). This compares to approximately 12 mrad radiation exposure from a chest x-ray and yearly exposure to 300 mrad from background radiation (Kelsey, Mettler & Sullivan, 1996). This method, is thus, not recommended in pregnant women and children and repeated use in research study settings with multiple trials (Jackson, Leahy, McGowan, Bluck, Coward & Jebb, 2004). Further disadvantages include the cost (Rose, 1979), the use of complex equipment by a skilled operator, and the time required to record a single observation, which has potential to result in inaccuracies particularly in patients who have rapid emptying (Sheiner, 1975).

The second most preferred method is the double sampling gastric aspiration technique of George (1968) as modified by Beckers, Rehrer, Brouns, Tenhoor & Saris, (1988). This method requires intubation of the stomach and the use of a non-absorbable dye, phenol red. The double sampling aspiration method of George (1968) advanced on early nasogastric methods of the serial test meal (Hunt & Spurrell, 1951) which was time consuming, requiring days to complete, and required daily repeated intubations to aspirate entire gastric contents at different time intervals. This current method where remaining gastric volumes are measured at regular intervals is based on the determination of dye concentration in small gastric samples aspirated before and after the addition of a known volume and concentration of dye to the gastric contents (Beckers *et al*., 1988; George, 1968). The modification of Beckers *et al*., (1988) allows the simultaneous determination of gastric secretion rate and volume that contribute to the total volume of gastric contents at any one point. This technique is reliable, can be highly accurate and has clinical applications (Sheiner, 1975; George, 1968). It is also relatively inexpensive compared to scintigraphy and can be used to measure gastric emptying characteristics even during exercise (Jeukendrup & Moseley, 2010; van Nieuwenhoven, Wagenmakers, Senden, Brouns & Brummer, 1999). Disadvantages, however, include the fact that the procedure is invasive and its use dependent on the ability of the person to swallow the tube. This can therefore limit subject recruitment. Furthermore, this technique can only be used for the assessment of liquid emptying, and possible

disturbances of normal physiology may be induced by intubation (Feinle, Kunz, Boesiger, Fried & Schwizer, 1999).

With the advancement and development of imaging techniques, methods such as magnetic resonance imaging (MRI) and real-time ultrasonography have been used more recently for the assessment of gastric emptying. Both of these techniques negate the disadvantages of radiation exposure and invasiveness. Gadolinium tetraazacyclododecane tetraacetic acid (Gd-DOTA) is the label of choice in MRI assessment, as it has been shown as the most stable contrast agent in the acidic conditions of the gastric environment, to adhere well to both liquids and solids, and is not easily absorbed (de Zwart & de Roos, 2010). This method of imaging has been shown to be reproducible with high inter-observer agreement (Carbone, Tanganelli, Capodivento, Ricci & Volterrani, 2010) and has been validated against scintigraphy for the measurement of liquids and mixed liquid-solid meals with correlations of 0.917 and 0.988, respectively (Feinle *et al*., 1999). Scans are performed in 3D and image slices are processed for areas of interest and volume calculated by multiplying the sum of the areas by the slice thickness (Feinle *et al*., 1999). A limitation of MRI is that gastric secretions need to be estimated by taking into account relative signal intensities of ex-vivo imaging of the test meal at different dilutions with the addition of hydrochloric acid (Szarka & Camilleri, 2009). A further drawback is that measurements are perfomed with the patient or volunteer in a right sided semi-supine position as the availability of seated MRI is uncommon (de Zwart & de Roos, 2010).

The measurement of gastric emptying using real-time ultrasound was first described by Bateman & Whittingham in 1982. Similar to the deduction of volume in MRI, a series of cross-sectional images (90° to the long axis of the stomach) are obtained at regular intervals to produce a three-dimensional representation of the stomach (Bateman & Whittingham, 1982). This technique is relatively inexpensive and the equipment is widely available (Szarka & Camilleri, 2009; Gentilcore, Hausken, Horowitz & Jones, 2006) and has been shown to hold good intra-observer and inter-observer agreement (Darwiche, Almer, Bjorgell, Cederholm & Nilsson, 1999) as well as correlate strongly with scintigraphy (Gentilcore *et al*., 2006). On the other hand, disadvantages of using ultrasound are that it is time consuming, requires a skilled operator and quality of images is impaired in people with a high body fat percentage and when there is excessive air in the stomach (Szarka & Camilleri, 2009;

Darwiche *et al*., 1999). Furthermore, it is not well suited for determining the emptying rate of solids.

A couple of indirect methods of gastric emptying assessment are also utilised which are based on the premise that measurement of changes in the concentration of either a pharmacological tracer or isotopic tracer in blood samples indicates absorption and thus gastric emptying rate. One such method is the paracetamol absorption test. This method involves simultaneous ingestion of the pharmacological substance with the test meal in doses ranging from 20 mg/kg body weight to 1.5 g and the collection of repeated blood samples (Willems, Quatero & Numans, 2001). Paracetamol concentration in the blood samples are then determined by gas liquid chromatography, or more recently developed fluorescence or enzymatic immunoassays and colorimetric assays. It is therefore an invasive method but relatively easy and inexpensive (Naslund *et al*., 2000). Since the absorption of paracetamol in the stomach is neglible (Clements, Heading, Nimmo & Prescott, 1978) serum paracetamol concentrations have been stated to reflect gastric emptying rate (Clements *et al*., 1978; Heading, Nimmo, Prescott & Toothill, 1973). The validity of the paracetamol absorption test appears only to apply with liquid emptying however, thus limiting the ability to use this technique for the measurement of solid food emptying. A systematic review of thirteen studies concluded gastric emptying of liquids or the liquid phase of test meals measured by paracetamol absorption was well correlated with scintigraphy; eight studies had a good correlation ($r > 0.6$), two a moderate correlation ($r = 0.45–0.6$) and three no correlation ($r < 0.45$) (Willems *et al*., 2001). However, the precision and reproducibility of the paracetamol absorption method can be questioned. Intrasubject variability for ten subjects measured on four separate occasions was reported by Petring & Flachs (1990) to be non-significant for all paracetamol absorption parameters. However, intrasubject coefficient of variations that can be calculated from the reported subject data are high with 15% for peak plasma concentration (C_{max}), 44% for time of peak plasma concentration (T_{max}), and 16% for 90 min area under curve (AUC). Furthermore, pharmokinetics of paracetamol absorption vary between and within individuals (Medhus, Lofthus, Bredesen & Husebye, 2001).

The deuterium tracer technique is a method used predominantly to measure ingested water uptake into the body pool but is also occasionally utilised as a proxy of gastric emptying assessment. This technique involves the ingestion of a test solution containing a trace amount (typically 5-12 g) of stable nonradioactive hydrogen isotope incorporated as

deuterium oxide (^2H_2O), also known as 'heavy water'. Collection and analysis of blood samples for ^2H accumulation then indicates the combination of absorption and gastric emptying rates (Lambert, Ball, Leiper & Maughan, 1999). Concentrations of ^2H_2O can then be analysed using a simple and inexpensive infrared spectrophotometry protocol (Lukaski & Johnson, 1985). Parameters of peak blood concentration (C_{max}) and time to reach peak blood concentration (T_{max}), as with the paracetamol absorption test, and accumulation rate are then utilised to indicate absorption and gastric emptying rates. A significant correlation of $r = 0.63$ has been reported between gastric emptying rate of carbohydrate solutions measured by the double sampling gastric aspiration technique and the rate at which deuterium oxide accumulated in plasma (Murray, Bartoli, Eddy & Horn, 1997). However, only 40% of the variation in plasma deuterium oxide accumulation could be attributed to gastric emptying rate (Murray *et al*., 1997) and thus does not provide a valid assessment of gastric emptying rate alone. Other disadvantages of this technique are that it is invasive and can only be used for liquid or liquid phase emptying.

An increasingly popular indirect method for the measurement of gastric emptying is the use of breath testing. This technique utilises the ^{13}C- stable isotope as a marker and was first introduced by Ghoos, Maes, Geypens, Mys, Hiele, Rutgeerts *et al.* (1993). It is based on the principle of rapid intestinal absorption and prompt hepatic oxidation of the incorporated label to $^{13}CO_2$, which subsequently appears in the breath, and assumes that these processes of absorption, oxidation and exhalation occur at a constant rate (Braden, 2009). Breath samples are collected at regular intervals and analysed for the ratio of $^{13}CO_2$:$^{12}CO_2$ using isotope ratio mass spectrometry or non-dispersive infra-red spectroscopy. The rate of isotopic enrichment of the breath is used to determine the rate of emptying of a meal (Sanaka & Nakada, 2010; Braden, 2009; Verbeke, 2009; Ghoos *et al*., 1993). Both liquid and solid emptying can be assessed separately by this method using different substrates; ^{13}C-acetate for liquid and semi-solid emptying and ^{13}C-octanoate for solid emptying. The ^{13}C-acetate breath test can also be used during exercise, though comparisons between exercise and rest conditions would be inaccurate due to alterations of absorption, oxidation and exhalation (van Nieuwenhoven *et al*., 1999). Breath testing has been validated against scintigraphy and gastric aspiration with significant correlation coefficients ranging from 0.76-0.95 and 0.82-0.94 reported for determined $T_{½}$ and T_{lag}, respectively (Braden, Peterknecht, Piepho, Schneider, Caspary, Hamscho *et al*., 2004; van Nieuwenhoven *et al*., 1999; Pfaffenbach, Schaffstein, Adamek,

Lee & Wegener, 1996; Braden, Adams, Duan, Orth, Maul, Lembcke *et al*., 1995; Ghoos *et al*., 1993). Although correlated, the absolute $T_{½}$ and T_{lag} values from the breath test are not directly comparable to those obtained using these alternative methods, however. This is because the terms $T_{½}$ and T_{lag} in breath testing indicates the time in which half of the total cumulative recovered dose of substrate has been metabolised when time is infinite and the time in which $^{13}CO_2$ excretion rate is at its maximum (Sanaka & Nakada, 2010; Ghoos *et al*., 1993). Application of this method in the clinical and research setting is very suitable as it is non-invasive, non-radioactive, safe, simple and effective (Braden, 2009).

1.5. FACTORS THAT AFFECT GASTRIC EMPTYING

Numerous factors have been shown to independently influence the rate of gastric emptying. Increasing the ingested volume of a liquid or solid meal proportionately increases the initial rate of emptying (Sanford, 1992; Costill & Saltin, 1974; Hunt & Knox, 1968; Hunt & MacDonald, 1954). Similarly, increasing the weight of a solid meal independently of energy content, and thus perhaps simultaneously increasing the volume of an ingested meal, also increases the rate of emptying (Moore, Christian, Brown, Brophy, Datz, Taylor *et al*., 1984). With specific regards to liquids, the volume of fluid in the stomach appears to be the most important determinant of emptying rate (Noakes, Rehrer & Maughan, 1991).

The energy content or density of an ingested solution or meal also affects the rate of emptying. Increasing the energy content and/or density of solutions (Vist & Maughan, 1994) and meals (Peracchi, Gebbia, Ogliari, Fraquelli, Vigano, Baldassarri *et al*., 2000; Hunt & Stubbs, 1975) slows gastric emptying. This is true regardless of the relative contributions of energy from fat, carbohydrate and protein (Hunt, 1980) which at isoenergetic amounts slow gastric emptying to the same degree (Hunt & Stubbs, 1975).

Increasing the concentration of an energy source in a meal results in a concurrent increase in osmolality (Vist & Maughan, 1995). Osmolality of a solution or meal has also been shown to independently affect gastric emptying rate. Increases in osmolality result in slowing of gastric emptying rate (Vist & Maughan, 1995; Costill & Saltin, 1974; Hunt & Knox, 1968). Furthermore, the effect of osmolality on the slowing of gastric emptying has been shown to be more marked at higher carbohydrate solution concentrations (Vist & Maughan, 1995). The osmolality of contents in the upper small intestine also strongly influences the rate of emptying (Hunt, 1960).

Other factors that have less extensively been shown to influence emptying include the pH of test meals, the temperature of drink solutions, body posture and blood glucose level. The greater the acidity of contents in the stomach the greater the slowing of emptying (Sanford, 1992; Hunt & Knox, 1972). Cold liquids at 4°C have been observed to empty more slowly compared to warm liquids at 37°C (Sanford, 1992), and lying in the supine position has also been reported to slow emptying compared with sitting or standing (Moore *et al*., 1988). Physiological increases of blood glucose levels to 8 mmol/L is also indicated to slow gastric emptying whilst hypoglycaemia accelerates emptying (Schvarcz, Palmer, Aman, Horowitz, Stridberg & Berne, 1997).

1.6. HORMONES INVOLVED IN THE REGULATION OF APPETITE AND FOOD INTAKE

An extensive list of hormones with important sensing and signalling roles in the regulation of hunger, satiety and food intake are derived from the gastrointestinal tract; the largest endocrine organ in the body (Karra & Batterham, 2010; Neary & Batterham, 2009). Many of these gastrointestinal peptides interact with the central nervous system via the gut-brain axis, acting on appetite centres of the hypothalamus and brain stem. The majority of gut hormones exhibit inhibiting effects on satiety and satiation. Satiety is the inhibition of hunger and further eating resulting from food consumption, and is influenced by postingestive and postabsorptive factors that act in the postprandial period to influence the length of inter-meal interval and/or the amount consumed during a subsequent meal (Blundell, Hill & Rogers, 1988). Satiation, on the other hand, is the short-term immediate process that controls meal size by terminating the period of eating and is primarily influenced by instant orosensory and cognitive influences, gastric distension and gut hormone secretion (Blundell *et al*., 1988). In addition, several hormones derived from the pancreas as well as adipose tissue have also been implicated to influence appetite. The major gut hormones involved in the regulation of appetite and food intake are discussed below.

1.6.1. Ghrelin

To date, ghrelin is the only gut hormone known to have orexigenic properties (Karra & Batterham, 2010; Huda, Wilding & Pinkney, 2006). Ghrelin is a 28 amino acid peptide produced and released from X/A-like cells of the gastric oxyntic glands of the fundus, and to

a lesser extent, the small intestine (Date, Kojima, Hosoda, Sawaguchi, Mondal, Suganuma *et al*., 2000; Kojima, Hosoda, Date, Nakazato, Matsuo & Kangawa, 1999). Initial discovery of this hormone revealed it as the natural ligand for the growth hormone secretagogue receptor (GHS-R) (Kojima *et al*., 1999), which are expressed predominantly in the pituitary and hypothalamus (Howard, Feighner, Cully, Arena, Liberator, Rosenblum *et al*., 1996). Ghrelin exists in two major molecular forms; acylated ghrelin, in which the third amino acid serine is covalently linked to a medium chain fatty acid octanoic acid, and des-acylated ghrelin, which has not undergone the post-translational modification to produce the former. The acylated form of ghrelin is considered as the biologically active peptide responsible for its orexigenic and growth hormone releasing actions (Huda *et al*., 2006; Kojima *et al*., 1999). Circulating ghrelin levels have been shown to increase with fasting and immediately prior to meals, before rapidly declining in concert with food consumption in both rodents and humans (Cummings, Frayo, Marmonier, Aubert & Chapelot, 2004; Cummings, Purnell, Frayo, Schmidova, Wisse & Weigle, 2001; Tschop, Smiley & Heiman, 2000; Wren, Small, Ward, Murphy, Dakin, Taheri *et al*., 2000). This pattern of release suggests a role of ghrelin in meal initiation (Moran, 2009; Cummings *et al*., 2004). The degree and length of postprandial ghrelin suppression is dose-dependent with energy intake or load with higher energy meals inducing greater suppression than that of less energy dense meals of similar volume (Callahan, Cummings, Pepe, Breen, Matthys & Weigle, 2004). Meals high in carbohydrates have also been shown to suppress ghrelin levels more than meals high in protein or fat (Erdmann, Topsch, Lippl, Gussmann & Schusdziarra, 2004). In addition, central and peripheral administration of exogenous ghrelin stimulates food intake in rats (Wren *et al*., 2000), and peripheral administration in humans has been shown to increase appetite and food intake. Wren and colleagues (Wren, Seal, Cohen, Brynes, Frost, Murphy *et al*., 2001) reported that peripheral administration of ghrelin in man resulted in a 28% increase in energy consumption during an *ad-libitum* buffet meal that was coupled with concomitant increases in subjective hunger scores. Ghrelin has also been suggested to play a role in long-term body mass regulation (Cummings & Shannon, 2003).

1.6.2. Glucagon like peptide-1 (GLP-1)

GLP-1 is a 30 amino acid peptide secreted from L-cells in the intestinal mucosa. It is synthesised by post-translation processing of pre-proglucagon into two forms; GLP-$1^{1\text{-}37}$ and

GLP-$1^{1\text{-}36\ amide}$, both of which demonstrate little biological activity (Chaudhri, Small & Bloom, 2006). Subsequent cleavage at the N-terminus produces the respective biologically active peptides GLP-$1^{7\text{-}37}$ and GLP-$1^{7\text{-}36\ amide}$, with GLP-$1^{7\text{-}36\ amide}$ being the main circulating form (Orskov, Rabenhoj, Wettergren, Kofod & Holst, 1994). The half-life of GLP-$1^{7\text{-}36\ amide}$ is less than 2 min as it is rapidly degraded by dipeptidyl peptidase IV (DPP-IV) into biologically inactive GLP-$1^{9\text{-}36}$ (Delzenne, Blundell, Brouns, Cunningham, De Graaf, Erkner *et al*., 2010). Secretion of this hormone occurs in a biphasic manner in response to food ingestion. Circulating levels increase within 10 to 20 min of eating with the first peak in circulating concentration occuring approximately 30 min following meal ingestion, and release is proportional to energy intake (Huda *et al*., 2006). A second smaller peak occurs several hours later and is thought to arise in response to nutrient stimulation in the large intestine (Delzenne *et al*., 2010). As such, GLP-1 has been suggested to play a minor role in satiation (Blundell & Naslund, 1999) but likely to have a major role in satiety by either prolonging the interval between meals or by reducing subsequent meal size (Feinle, O'Donovan & Horowitz, 2002). The ingestion of carbohydrates produces a greater and more rapid secretory response than fats or proteins (Herrmann, Goke, Richter, Fehmann, Arnold & Goke, 1995; Elliott, Morgan, Tredger, Deacon, Wright & Marks, 1993). GLP-1 exerts its effects via GLP-1 receptors (GLP-1R) which are widely expressed throughout the central nervous system (CNS) and on peripheral tissues (Bullock, Heller & Habener, 1996). Both direct intracranial and peripheral administration in rodents have been shown to potently reduce food intake (Turton, O'Shea, Gunn, Beak, Edwards, Meeran *et al*., 1996). In humans, a meta-analysis by Verdich and colleagues, (Verdich, Flint, Gutzwiller, Naslund, Beglinger, Hellstrom *et al*., 2001) concluded that peripheral administration of GLP-1 reduces energy intake dose dependently by 11.7%. GLP-1 is also known as an incretin hormone, whereby approximately 50% of the glucose-stimulated rise in postprandial insulin concentration is attributed to this hormone whilst inhibiting glucagon secretion (Feinle *et al*., 2002).

1.6.3. Glucose dependent insulinotropic polypeptide (GIP)

GIP, also known as gastric inhibitory peptide, is released from K-cells in the mucosa of the duodenum and proximal jejunum (Feinle *et al*., 2002). It is released in response to carbohydrate, fat and protein intake (Herrmann *et al*., 1995). Similar to GLP-1, it is an incretin hormone, enhancing postprandial glucose-stimulated insulin release. Evidence for

the involvement of GIP in the regulation of appetite and food intake is limited and unknown, however (Chaudhri *et al*., 2006). It has been shown not to affect food intake in rodents when administered intracranially or peripherally (Garlicki, Konturek, Majka, Kwiecien & Konturek, 1990; Lorenz, Kreieksheimer & Smith, 1979) and no relationship has been found with its effect on appetite in humans (Lavin, Wittert, Andrews, Yeap, Wishart, Morris *et al*., 1998). Despite this, a role of GIP in appetite regulation and food intake mediated via its incretin effects or other interactions with other gut hormone pathways is perhaps not yet totally dismissible.

1.6.4. Peptide tyrosin tyrosin (PYY)

Peptide tyrosine tyrosine, commonly abbreviated to peptide YY, is a 36 amino acid peptide. Like GLP-1, it is synthesised and released from the L-cells of the intestinal muscosa. Two main forms of PYY have been described; PYY^{1-36} and its truncated bioactive 34 amino acid product PYY^{3-36} formed through enzymatic cleavage by DPP-IV (Mentlein, Gallwitz & Schmidt, 1993). This latter truncated hormone is the predominant circulating form of the peptide in both fasted and fed states (Batterham, Heffron, Kapoor, Chivers, Chandarana, Herzog *et al*., 2006; Korner, Inabnet, Conwell, Taveras, Daud, Olivero-Rivera *et al*., 2006). PYY^{3-36} exerts its effects via the neuropeptide Y2 receptor (NPY2R) which are widely present in the CNS and hypothalamus (Karra & Batterham, 2010; Cummings & Overduin, 2007) and gastrointestinal tract (Mo & Wang, 1994). Circulating concentrations of PYY are suppressed in the fasted state and increase within 30 min in response to nutrient ingestion, typically peaking between 1-2 h postprandially before maintaining a plateau for several hours (Adrian, Savage, Sagor, Allen, Bacaresehamilton, Tatemoto *et al*, 1985). Release of PYY is dose-dependently related to energy intake and also varies with food consistency and maconutrient intake (Huda *et al*., 2006). Fat particularly stimulates PYY release compared with carbohydrate and protein meals of similar energy content (Adrian et al., 1985; Taylor, 1985). Peripheral administration of PYY^{3-36} has been shown to reduce food intake in rodents and humans (Batterham, ffytche, Rosenthal, Zelaya, Barker, Withers *et al*., 2007; Sloth, Davidsen, Holst, Flint & Astrup, 2007; le Roux, Batterham, Aylwin, Patterson, Borg, Wynne *et al*., 2006; Chelikani, Haver & Reidelberger, 2004; Batterham, Cowley, Small, Herzog, Cohen, Dakin *et al*., 2002). In healthy normal weight humans, this administration led to a 33% reduction in energy intake, a shortening in the duration of food intake and a decrease in

hunger ratings that persisted for up to 12 hours post infusion (Batterham *et al*., 2002). Others have also shown that bolus doses or slow intravenous infusions of PYY reduce the size of meals eaten and increases the between-meal interval length (Chelikani, Haver & Reidelberger, 2005; Moran, Smedh, Kinzig, Scott, Knipp & Ladenheim, 2005). Intra-cerebral administration in rodents in particular areas of the brain have also been shown to reduce food intake (Batterham *et al*., 2002). A role in long term body weight regulation has also been implicated for PYY^{3-36} (Karra & Batterham, 2010).

1.6.5. Pancreatic polypeptide (PP)

A member of the PP-fold peptide family along with PYY and neuropeptide Y, PP is a 36 amino acid produced and secreted by F-cells predominantly found in the peripheries of the pancreatic islets of Langerhans and to a lesser extent in the colon (Ekblad & Sundler, 2002). The actions of PP are mediated by Y4 and Y5 receptors for which the hormone has greatest affinity (Larhammar, 1996). The major sites of action of PP are believed to be the hypothalamus and the brain stem, which are regions where the blood brain barrier are absent (Karra & Batterham, 2010; Huda *et al*., 2006). As with GLP-1, circulating levels of PP increase following nutrient ingestion in a biphasic manner proportional to the energy load and remain elevated up to 6 hours postprandially (Track, McLeod & Mee, 1980). Administration of PP centrally has been reported to increase food intake in rodents (Asakawa, Inui, Yuzuriha, Ueno, Katsura, Fujimiya *et al*., 2003). In addition, peripheral administration of PP has been shown to reduce food intake in normal weight and genetically obese (ob/ob) rodents (Liu, Semjonou, Murphy, Ghatei & Bloom, 2008; Neary, McGowan, Monteiro, Jesudason, Ghatei & Bloom, 2008; Asakawa, Uemoto, Ueno, Katagi, Fujimiya, Fujino *et al*., 2006; Asakawa *et al*., 2003) and in humans (Batterham, le Roux, Cohen, Park, Ellis, Patterson *et al*., 2003). The authors of this latter study observed a reduction in food intake by 22% during an *ad libitum* buffet meal.

1.6.6. Cholecystokinin (CCK)

Cholecystokinin was the first gut hormone shown to exhibit influences on appetite (Gibbs, Young & Smith, 1973). Multiple molecular forms of this hormone with amino acids ranging from 8 to 58 exist. The most widely studied and considered forms of CCK in relation to appetite and food intake regulation are CCK-8 and -33 (Delzenne *et al*., 2010). CCK is

synthesised and released mainly from I-cells of the intestinal mucosa in the duodenum and proximal jejunum (Delzenne *et al*., 2010; Moran, 2009; Huda *et al*., 2006). It is also produced by neurons in the GI tract and is widely distributed in the brain, where it functions as a neurotransmitter (Barden, Merand, Rouleau, Moore, Dockray & Dupont, 1981; Hutchison, Dimaline & Dockray, 1981). CCK acts on both CCK-1 and CCK-2 receptors, which were formerly known as CCK-A and -B, respectively for its localisation to the 'alimentary' and 'brain' (Neary & Batterham, 2009). Appetite regulatory effects are attributed to CCK-1 receptor mediation (Asin, Bednarz, Nikkel, Gore & Nadzan, 1992), which are expressed on the gallbladder, pancreas, stomach, pyloric sphincter, vagal afferent fibres and the dorsomedial hypothalamus (Delzenne *et al*., 2010; Neary & Batterham, 2009; Chaudhri *et al*., 2006; Moran, 2000). CCK is secreted most greatly in response to fat, followed by protein then carbohydrate (Mo & Wang, 1994; Liddle, Goldfine, Rosen, Taplitz & Williams, 1985). Levels of circulating CCK rise over 10-30 min following meal initiation and declines gradually to baseline values in 3-5 h (Liddle *et al*., 1985). Intravenous infusion of small doses of CCK-8 in humans has been shown to reduce food intake and increase satiety (Stacher, Steinringer, Schmierer, Schneider & Winklehner, 1982; Kissileff, Pisunyer, Thornton & Smith, 1981). Furthermore, central administration in rodents has also largely been shown to decrease feeding (Huda *et al*., 2006).

1.6.7. Oxyntomodulin

Similar to GLP-1, oxyntomodulin (OXM) is produced by post-translation processing of pre-proglucagon. The 37 amino acid peptide is released by the same intestinal L-cells that release GLP-1 and PYY in response to food ingestion in proportion to energy intake (Lequellec, Kervran, Blache, Ciurana & Bataille, 1992; Ghatei, Uttenthal, Christofides, Bryant & Bloom, 1983). As with CCK, release of OXM is predominantly stimulated by fatty acids in the gut lumen (Read, McFarlane, Kinsman, Bates, Blackhall, Farrar *et al*., 1984). OXM is also found expressed in the CNS and pancreas (Huda *et al*., 2006). Central as well as peripheral administration of OXM in rats has been shown to reduce food intake (Dakin, Small, Batterham, Neary, Cohen, Patterson *et al*., 2004; Dakin, Gunn, Small, Edwards, Hay, Smith *et al*., 2001). The same outcome has also been shown in humans. Intravenous infusion in normal weight humans resulted in a 19% reduction in immediate energy intake as well as inhibiting food intake over the subsequent 12 h (Cohen, Ellis, le Roux, Batterham, Park,

Patterson *et al*., 2003). In addition, OXM has been shown to suppress circulating ghrelin concentrations by approximately 15% and 44% in rats and humans, respectively (Dakin *et al*., 2004; Cohen *et al*., 2003). The effects of OXM appear to be mediated via the GLP-1R (Baggio, Huang, Brown & Drucker, 2004). However, as it's affinity to the receptor is much weaker than GLP-1, other receptors or even a unique receptor to OXM yet to have been identified may be involved (Fehmann, Jiang, Schweinfurth, Wheeler, Boyd & Goke, 1994).

1.6.8. Amylin

Amylin is a 37 amino acid peptide that is co-released with insulin at a molar ratio of 100:1 (insulin:amylin) from pancreatic beta cells in response to food ingestion (Neary & Batterham, 2009; Butler, Chou, Carter, Wang, Bu, Chang *et al*., 1990). Concentrations rise rapidly before peaking within 1 h and remain elevated for up to 4 h postprandially (Koda, Fineman, Rink, Dailey, Muchmore & Linarelli, 1992). It's anorectic effects of decreasing meal size have been demonstrated in rodents through both central and peripheral administration (Lutz, Geary, Szabady, Delprete & Scharrer, 1995). Studies in diabetic and non-diabetic obese humans have also implicated a role of amylin in reducing free-choice food intake by using pramlintide, a synthetic amylin analogue, (Chapman, Parker, Doran, Feinle-Bisset, Wishart, Strobel *et al*., 2005). Receptors to which amylin binds have been described within the CNS which include the hypothalamus and area postrema of the brainstem (Lutz, Mollet, Rushing, Riediger & Scharrer, 2001).

1.6.9. Other important hormones in the regulation of appetite

Leptin is a peptide hormone produced and secreted predominantly from white adipose tissue (Zhang, Proenca, Maffei, Barone, Leopold & Friedman, 1994), now known to be an important active endocrine organ, as well as from the gastric epithelium although in smaller amounts (Bado, Levasseur, Attoub, Kermorgant, Laigneau, Bortoluzzi *et al*., 1998). Leptin, a product of the *ob* gene, has several actions, including roles in energy homeostasis and neuroendocrine and immune functions (Stanley, Wynne, McGowan & Bloom, 2005). Concentrations of leptin are strongly correlated with mass of adipose tissue (Maffei, Halaas, Ravussin, Pratley, Lee, Zhang *et al*., 1995) and are relatively insensitive to food intake (Stanley *et al*., 2005). Early studies such as that by Ahima and colleagues (Ahima, Prabakaran, Mantzoros, Qu, Lowell & Maratos-Flier *et al*., 996) have reported that both

central and peripheral administration of leptin reduces food intake spontaneously and following fasting. Leptin is thus considered to exert a long term regulatory role in appetite and food intake (Hellstrom, Geliebter, Naslund, Schmidt, Yahav, Hashim *et al*., 2004).

Adiponectin is also, but solely, produced and secreted from adipose tissue. In contrast to leptin, levels of adiponectin are negatively correlated to BMI (Matsubara, Maruoka & Katayose, 2002). Circulating concentrations of adiponectin are also not affected by food intake (Hotta, Funahashi, Arita, Takahashi, Matsuda, Okamoto *et al*., 2000) and it is considered as a long term regulator of food intake (Hellstrom *et al*., 2004).

Insulin is produced and secreted from beta cells of the pancreatic islets of Langerhans. Similar to leptin, it is positively correlated with long term energy balance (Woods, Decke & Vasselli, 1974; Bagdade, Bierman & Porte, 1967). Circulating concentrations of insulin increase rapidly after a meal (Polonsky, Given & Vancauter, 1988), particularly in response to carbohydrates. The role of insulin in the regulation of appetite remains unclear, however. Studies in animals have indicated euglycaemic hyperglycaemia decreases food intake (Air, Strowski, Benoit, Conarello, Salituro, Guan *et al*., 2002; Woods, Stein, McKay & Porte, 1984; Nicolaidis & Rowland, 1976) whilst a study in healthy humans have shown physiological concentrations of insulin are unlikely to play a role in satiation and short term appetite control (Chapman, Goble, Wittert, Morley & Horowitz, 1998). Indirect mechanisms in which blood insulin concentrations influence appetite by acting in synergy with other hormones or factors such as blood glucose concentration remains a plausible premise (Feinle *et al*., 2002).

1.7. SUMMARY TABLE OF HORMONES WITH A ROLE IN GASTRIC EMPTYING

As previously mentioned, the process of gastric emptying is stimulated and affected by humoral activity and signals. The majority of aforementioned gut hormones implicated in the regulation of appetite, food intake and energy balance have also been shown to exert effects on gastric emptying (Hellstrom *et al*., 2006; Mo & Wang, 1994). The effect of these hormones and several others known to influence gastric emptying are summarised in Table 1.

Table 1. Major hormones involved in the regulation of appetite, food intake, and gastric emptying

Hormone	Primary synthesis and secretion site	Function in appetite and food intake	Target organs in appetite control	Function on gastric emptying	Gastric emptying reference(s)
Ghrelin (acylated)	Gastric fundus (X/A-like cells) Small intestine	Initiates meal intake Increases appetite and food intake Long term regulation of body weight	Vagus Brainstem Hypothalamus	Promotes gastric emptying	Levin *et al*., 2006 Murray *et al*., 2005 Asakawa *et al*., 2001
Glucagon-like peptide-1	Distal small intestine (L-cells)	Reduces energy intake Increases satiety Increases satiation	Vagus Brainstem Hypothalamus	Strongly inhibits gastric emptying (‘ileal brake’)	Wettergren *et al*., 1993 Wishart *et al*., 1998
Glucose dependent insulinotropic polypeptide	Duodenum and proximal jejunum (K-cells)	Unclear	?	Equivocal (promotes or no effect)	Edholm *et al*., 2010 Meier *et al*., 2004
Peptide YY (3-36)	Distal small intestine (L-cells)	Reduces food intake Increases satiety Increases satiation	Vagus Brainstem Hypothalamus	Strongly inhibits gastric emptying (‘ileal brake’)	Chen *et al*., 1996 Taylor, 1993 Savage *et al*., 1987
Pancreatic polypeptide	Pancreatic islets of Langerhans (F-cells)	Reduces food intake Increases satiety Increases satiation	Vagus Brainstem	Equivocal effect on gastric emptying	Adrian *et al*., 1981 Batterham *et al*., 2003 Kojima *et al*., 2007
Cholecystokinin	Duodenum and proximal jejunum (I-cells	Reduces food intake Increases satiation	Vagus nerve Brainstem Hypothalamus	Inhibits gastric emptying	Kelly, 1980
Oxyntomodulin	Distal small intestine (L-cells)	Reduces food intake Increases satiety Increases satiation	Hypothalamus	Inhibits gastric emptying	Schjoldager *et al*., 1989
Amylin	Pancreatic islets of Langerhans (beta cells)	Increases satiety Increases satiation	Brainstem Hypothalamus	Inhibits gastric emptying	Young *et al*., 1996
Gastrin	Gastric antrum (G-cells)	No known effect	?	Inhibits gastric emptying	Kelly, 1980
Secretin	Duodenum (S-cells)	No known effect	?	Inhibits gastric emptying	Valenzuela *et al*., 1981
Somatostatin	Pancreas and intestines (D-cells)	No known effect in humans	?	Inhibits gastric emptying	Reichlin, 1983
Motilin	Duodenum and jejunum (M-cells)	No known effect	?	Promotes gastric emptying	Schmid *et al*., 1991 Christofides *et al*., 1979

1.8. ROLE OF GENETICS IN OBESITY AND GASTRIC EMPTYING

With recent developments and advances in human gene mapping and enhanced molecular techniques, it has become much easier for geneticists to investigate associations or linkage between genetic variation and obesity or appetite. There are two main methods of approach in such studies. One is the candidate gene approach where genes with potential association or involvement in a disease trait are selected and a small number of variants within the gene investigated for association (Sudbery & Sudbery, 2009). Although this approach offers high statistical power, it is however limited by current knowledge of biology and disease mechanisms (Amos, Driscoll & Hoffman, 2011; Sudbery & Sudbery, 2009). The second, which has become increasingly utilised, is the genome wide association study approach in which a large number of single nucleotide polymorphisms (SNP) that span across the human genome are scanned. Tagging SNPs, which are the minimum number of SNPs required to capture the common haplotype variation present, are often used to identify loci in which a causative variant resides (Xia & Grant, 2013).

Research into the genetics of obesity has predominantly focussed on monogenic or syndromic obesity in the past (Walley, Blakemore & Froguel, 2006). Following the discovery of the mouse obesity ob/ob gene and its human homologue product leptin (Zhang *et al*., 1994), rare mutations in genes within the leptin-melanocortin pathway have been identified to cause monogenic forms of obesity. These include the genes that encode leptin (Mammes, Betoulle, Aubert, Herbeth, Siest & Fumeron, 2000; Li, Reed, Lee, Xu, Kilker, Sodam *et al*., 1999; Montague, Farooqi, Whitehead, Soos, Rau, Wareham *et al*., 1997), leptin receptor (LEPR) (Clement, Vaisse, Lahlou, Cabrol, Pelloux, Cassuto *et al*., 1998), alpha-melanocortin 4 receptor (MC4R) (Vaisse, Clement, Durand, Hercberg, Guy-Grand & Froguel, 2000; Yeo, Farooqi, Aminian, Halsall, Stanhope & O'Rahilly, 1998) pro-opiomelanocortin (POMC) (Krude, Bibermann, Luck, Horn, Brabent & Gruters, 1998), and prohormone convertase-1 (Jackson, Creemers, Phagi, Raffin-Sanson, Sanders, Montague *et al*., 1997). A number of rare syndromes caused by both autosomal and x-linked genetic defects or abnormalities are also characterised by obesity and are associated with signs of hypothalamic dysfunction that result in hyperphagia (Bell, Walley & Froguel, 2005). Examples of these include Prader Willi Syndrome, which is usually caused by a paternally inherited deletion at the chromosome region 15q11.2-q12, Pseudohypoparathyroidism type 1A syndrome, which is caused by a maternally inherited mutation in the GNAS1 gene that encodes the alpha-subunit of the Gs protein, and Bardet-Biedl syndrome, an autosomal recessive condition where any number of mutations at

various loci occur (Beales, Elcioglu, Woolf, Parker & Flinter, 1999). More recently, however, with the advances in genomic research and the epidemic increase in obesity, greater emphasis has been placed on the genetics of common, non-syndromic obesity. It is widely accepted that common obesity contains a polygenetic component that enhances susceptibility to severe weight gain and development of obesity in today's 'obesegenic environment' (Cheung & Mao, 2012; Bell *et al*., 2005; Friedman, 2003). This predisposition is theorised in the 'thrifty gene hypothesis' (Neel, 1962) to have previously been advantageous in past populations who experienced regular periods of starvation (Bell *et al*., 2005). Furthermore, despite exposure to the same obesegenic environments considerable individual variation in body mass exists among populations (Farooqi, 2011). The earliest evidence of a genetic component to common obesity came from twin studies. Stunkard, Foch & Hrubec (1986) reported high body mass (BM) and BMI heritability estimates of 78% and 77%, respectively at age 20 y, which then increased to 81% and 84% respectively at 25-year follow up with monozygotic and dizygotic twins. These heritability estimates were additionally supported with significant positive correlations between BM classes of adoptees and BMI of biological parents but not adoptive parents (Stunkard, Sorensen, Hanis, Teasdale, Chakraborty, Schull *et al*., 1986), and also BM of adult adoptee siblings (Sorensen, Price, Stunkard & Schulsinger, 1989). Further studies on monozygotic and dizygotic twins showed correlations in BM were approximately 0.7 and 0.2, respectively, regardless of whether they were raised apart or together (Stunkard, Harris, Pedersen, & McClearn, 1990). Agreements in fat mass have also been reported to range from 70-90% in monozygotic twins and 35-45% in dizygotic twins (Xia & Grant, 2013; Bell *et al*., 2005; Farooqi & O'Rahilly, 2005; Hebebrand, Friedel, Schauble, Geller & Hinney, 2003).

An increasing number of genetic variants within the genes coding for gastrointestinal hormones and their receptors are being associated with obesity phenotypes and appetite and food intake regulation. Two hormones with the largest amount of evidence in support for an influence of genetics are ghrelin and PYY. Several SNPs within the ghrelin gene have been associated with obesity. For example, the -501A/C SNP in the promoter region of the gene has been associated with BMI and waist circumference (Vartiainen, Kesaniemi & Ukkola, 2006). However, it had not been associated with fasting ghrelin concentrations (den Hoed, Smeets, Veldhorst, Nieuwenhuizen, Bouwman, Heidema *et al*., 2008; Vartiainen *et al*., 2006). On the other hand the variant 152G>A (G346A) which causes an amino acid change Arg51Gln has been shown associate with lower fasting plasma ghrelin levels (Poykko, Kellokoski, Horkkp, Kauma, Kesaniemi &

Ukkola, 2003; Ukkola, Ravussin, Jacobson, Snyder, Chagnon, Sjostrom *et al*., 2001) and also later onset of obesity (Ukkola *et al*., 2001). In addition, another variant 214C>A (C408A) that causes a Leu72Met amino acid change has also been associated with earlier onset of obesity (Ukkola *et al*., 2001). A number of SNPs and haplotypes within the GHS-R gene have also been reported to be associated and involved with the pathogenesis of obesity with increased risk of obesity ranging between 41% and 56% with the presence of the minor allele for the SNPs (Baessler, Hasinoff, Fischer, Reinhard, Sonnenberg, Olivier *et al*., 2005). Fasting plasma PYY concentrations have been shown to be lower with the uncommon SNP at A726C and Glu62Pro amino acid change in the PYY gene. Functional analysis in mice revealed the minor allele variant 62Pro resulted in greater food intake (Ahituv, Kavaslar, Schackwitz, Ustaszewska, Collier, Hebert *et al*., 2006). In addition, the 215G>C SNP causing an Arg72Thr change results in higher fasting PYY levels by 20% and lower risk of overweight and obesity (Torekov, Larsen, Glumer, Borch-Johnsen, Jorgenson, Holst *et al*., 2005). Other variants in the genes for PYY (Shih, Wang, Chiron, Wen, Nievergelt, Mahata, Khandrika *et al*., 2009; Siddiq, Gueorguiev, Samson, Hercberg, Heude, Levy-Marchal *et al*., 2007; Ma, Tataranni, Hanson, Infante, Kobes, Bogardus & Baier, 2005), NPY2R (Siddiq *et al*., 2007; Ma *et al*., 2005; Hung, Pirie, Luan, Lank, Motala, Yeo *et al*., 2004), CCK (de Krom, van der Schouw, Hendriks, Ophoff, van Gils, Stolk *et al*., 2007), CCK-1 receptor (Funakoshi, Miyasaka, Matsumoto, Yamamori, Takiguchi, Kataoka *et al*., 2000), and GLP-1R (Li, Tiwari, Lin, Allison, Chung, Leibel, *et al*., 2014) have also been reported to be associated with obesity phenotype measures.

Despite advances and an increasing number of associations between genetic variants and obesity susceptibility being identified, the combined results of all these linkage, candidate gene and genome wide association study approaches have explained only a small amount of the variance in BMI, suggesting there are still many genetic findings to be made (Xia & Grant, 2013). As many of the gastrointestinal hormones involved in appetite regulation also play a role in the regulation of gastric emptying, it is possible that genetic variations in gut hormones previously associated with obesity may be mediated through their effects on gastric emptying rate. In addition, other genetic variations not yet discovered or investigated, may explain the inter-individual variation in gastric emptying rate since part of the variability in postprandial responses of gut hormones has previously been shown to be explained by genetic variation (den Hoed *et al*., 2008). These authors have shown acylated ghrelin response, PYY response and perceived hunger to be associated with SNPs in the genes coding for PYY and LEPR, ghrelin and ghrelin receptor, and neuropeptide Y and ghrelin receptor, respectively (den Hoed *et al*., 2008).

1.9. DIET, GASTRIC EMPTYING, APPETITE AND FOOD INTAKE

The increasing prevalence of overweight and obesity, resulting from a consequence of positive energy balance where energy intake exceeds expenditure, continues to present a substantial burden on healthcare costs across the world. As a result, much research has focussed on the role of gut-derived hormones in regulating subjective feelings of appetite and satiety, and how interventions may affect the secretion of these hormones. As highlighted, the majority of these hormones have concomitant influences on gastric emptying. There appears, therefore, to be an intrinsic link between the regulation of gastric emptying and the regulation of appetite, with the former having been suggested as a possible important determinant of the latter (Delzenne *et al*., 2010). This integrated mechanism is highly favourable and realistic particularly as gastric distension is a potent satiety signal (Sanford, 1992). As different foods or macronutrients differentially affect the response profile of various hormones, an understanding of the regulation of gastric emptying and the relative roles of each associated hormone would potentially facilitate the development of dietary interventions aimed at suppressing or stimulating the secretion of specific hormones and ultimately modulating subjective feelings of appetite and energy intake.

1.9.1. Carbohydrates, appetite and food intake

Carbohydrates are an essential component of our diet, providing the majority of our dietary energy intake. Whether ingested orally or administered directly into the stomach or small intestine, carbohydrates reduce subsequent food intake (Feinle *et al*., 2002). Mechanisms that mediate this effect include gut hormone secretion in response to interaction with nutrient receptors in the gastrointestinal tract and also acute changes in blood glucose concentration which affect gastrointestinal function (Feinle *et al*., 2002). Different types of carbohydrate exist in our food and drink. Some of these are in the form of simple sugars, the most common being the monosaccharides glucose and fructose, and the disaccharide sucrose (table sugar) which is composed of one glucose molecule and one fructose molecule. It has been suggested that different types of carbohydrate or sugars may vary in their effects on food intake (Feinle *et al*., 2002). Recent research interests in carbohydrates and satiety have focused on fructose and its possible role in the pathogenesis of obesity and the metabolic syndrome.

1.9.1.1. Hepatic metabolism of fructose

Much of the attention on fructose has revolved around its central metabolic differences to glucose. Fructose and glucose are both hexose sugars with identical chemical formula $C_6H_{12}O_6$. The chemical structure of fructose differs from glucose, however, as it has a keto group in position two of its carbon chain instead of an aldehyde group at position one (Tappy & Le, 2010). Although fructose metabolism is closely tied to that of glucose metabolism and they converge within the glycolytic pathway (Feinman & Fine, 2013), there are some fundamental differences in how they are metabolised in the liver.

The metabolism of glucose involves an initial step of phosphorylation by glucokinase into glucose-6-phosphate before isomerization to fructose-6-phosphate. Glucokinase has a high Michaelis constant (K_m) to glucose such that the rate of glucose phosphorylation varies in concordance with changes in portal glucose concentration (Tappy & Le, 2010). The enzyme phosphofructokinase then catalyzes the conversion of fructose-6-phosphate to fructose-1,6-diphosphate, a step that is regulated by negative feedback by adenosine triphosphate (ATP) and citrate. Fructose-1,6-diphosphate is then split into the interconvertable triose phosphates glyceraldehyde-3-phosphate and dihydroxyacetone-phosphate of which the former is converted to pyruvate in the mitochondria and enters the Krebs cycle as acetyl-coA (Elliott, Keim, Stern, Teff & Havel, 2002).

Fructose on the other hand is initially rapidly phosphorylated into fructose-1-phospate catalysed by fructokinase which has a low K_m to fructose that results in efficient enzyme activity even at low concentrations of fructose. Fructose-1-phospate is then cleaved into the triose phosphates glyceraldehyde and dihydroxyacetone-phosphate by the action of aldolase B (Elliott *et al*., 2002; Mayes, 1993; Hallfrisch, 1990). These steps are not under negative feedback control and fructose metabolism is thus considered to bypass the major control point by which carbon from glucose enters glycolysis (Elliott *et al*., 2002). The unregulated production of triose phosphates then serves several pathways. Subsequent phosphorylation of both trioses to form glyceraldehyde-3-phosphate enables the production of pyruvate and subsequently acetyl-coA in the mitochondria for oxidisation in the Krebs cycle according to energy demands. However, as the synthesis of trioses exceeds the capacity of the liver to oxidise them, an amount of citrate is exported back into the cytosol for the formation of long chain fatty acids (Mayes, 1993). In addition, about a quarter of the triose phosphates that yield pyruvate are subsequently converted into lactate that is then released into the systemic circulation (Sun & Empie, 2012; Tappy & Le, 2010). Furthermore, a substantial amount of approximately 30-57% undergoes gluconeogenesis

for conversion to glucose (Sun & Empie, 2012; Delarue, Normand, Pachiaudi, Beylot, Lamisse & Rue, 1993) and at least 15% is estimated to convert to glycogen (Tappy & Le, 2010). Lastly, a small amount of approximately 1% serves as the backbone for triacylglycerol synthesis through de novo lipogenesis (Sun & Empie, 2012; Tappy & Le, 2010). Another unique feature of fructose metabolism is the production of uric acid. The depletion of ATP in the efficient phosphorylation of fructose upon entering the hepatocyte results in increased degradation of nucleotides to uric acid. An increased concentration of uric acid has been shown to be an independent risk factor for obesity, renal disease and cardiovascular diseases (Johnson, Segal, Sutin, Nakagawa, Feig, Kang *et al*., 2007).

1.10. OBJECTIVES OF THIS THESIS

The aims of this thesis were to determine some of the hormonal and genetic influences on gastric emptying characteristics in humans and to investigate the instrinsic link between the regulation of gastric emptying and the regulation of appetite and food intake with particular focus on dietary sugar and fructose ingestion.

This was achieved through the following research study objectives:

1. To investigate the gastric emptying characteristics, circulating gut hormone profiles and appetite responses to different oral carbohydrate solutions in humans.
2. To investigate the effect of increased dietary consumption of fructose on gastric emptying, circulating gut hormone profiles and appetite responses in humans.
3. To investigate the influence of gastric emptying rate on the hepatic metabolism of fructose
4. To investigate the influence of GLP-1R genetic variation on gastric emptying rate of a glucose solution in humans.

2. GENERAL METHODS

2.1. PRELIMINARY/FAMILIARISATION VISITS

All participants reported to the laboratory for a preliminary familiarisation visit before each study. Height was measured to the nearest 0.1 cm using a wall mounted stadiometer and BM was measured to the nearest 0.01 kg using electronic scales (GFK 150; Adam Equipment Co. Ltd., Milton Keynes, UK). Body fat percentage was estimated using a hand-held bioelectrical impedance device (Omron BF306; Kyoto, Japan). In addition, participants were familiarised with the gastric emptying breath sampling technique and the appetite assessment both detailed below to be used during the experimental trials. Furthermore, participants who had not previously participated in any studies involving fructose consumption completed a fructose tolerance test before further participation by consuming a 600 mL solution containing 36 g of fructose. This procedure was used to ensure that no adverse effects would be experienced due to unknown malabsorption during experimental trials.

2.2. PRE-TRIAL STANDARDISATION

In the 24 h preceding each experimental trial, participants were asked to refrain from alcohol and caffeine ingestion as well as the performance of strenuous physical activity. Participants were also asked to attend the laboratory in the morning following an overnight fast from 2100 h, with the exception of drinking 500 mL of water approximately 90 min prior to arrival at the laboratory. This was in an effort to ensure euhydration upon arrival and a consistent level of hydration status. All studies involving multiple experimental trials were conducted in a randomised single-blind crossover fashion and all experimental trials commenced between 0730 and 1030 h.

2.3. GASTRIC EMPTYING MEASUREMENT AND ANALYSIS

Gastric emptying characteristics were assessed in these studies using the non-invasive ^{13}C breath test method. This method was utilised due to its suitability for repeated testing, its non-invasiveness and its sufficiently high validity and reliability when compared with other methods. Test drink solutions ingested in all studies contained 100 mg of [^{13}C]sodium acetate (1-13C, 99%) (Cambridge Isotope Laboratories Inc., Andover MA, USA). Prior to ingestion of the test solution, a basal end-expiratory breath sample was collected. Further end-expiratory breath samples were collected at 10 min intervals for a total of 60 min following drink ingestion. On each occasion, breath samples were collected into a 100 mL foil bag by exhalation through a one-way valve mouthpiece (Wagner Analyzen-Technik, Bremen, Germany). Bags were then sealed with a plastic stopper and stored for later

analysis. A 1 h sampling period is deemed sufficient particularly when utilising the parameter T_{lag} as it occurs within 1 h post- ingestion (van Nieuwenhoven *et al*., 1999; Braden *et al*., 1995) due to the rapidity of liquid emptying compared to semi-solid or solid food ingestion which are normally assessed over a 4 h period. Furthermore, a small investigation conducted in our laboratory with eleven participants shows that the results from sampling every 10 min for a period of 1 h correlate moderately ($T_{½}$; r = 0.40, P = 0.143) and very strongly (T_{lag}; r = 0.85, $P < 0.01$) with the results from sampling for a period of 4 h. Previous studies have also used a sampling period of 60 min (Jeukendrup & Moseley, 2010; van Nieuwenhoven *et al*., 1999) and some even less with 45 min (Psichas, Little, Lal & McLaughlin, 2012; Little, Gopinath, Patel, McGlone, Lassman, D'Amato *et al*., 2010; Little, Gupta, Case, Thompson & McLaughlin, 2009).

Breath samples were analysed by non-dispersive IR spectroscopy (IRIS, Wagner Analyzen-Technik, Bremen, Germany) for the ratio of $^{13}CO_2$:$^{12}CO_2$. The difference in the ratio of $^{13}CO_2$:$^{12}CO_2$ from baseline breath to post-ingestion breath samples are expressed as delta over baseline (DOB). Half emptying time ($T_{½}$) and time of maximum emptying rate (T_{lag}) were calculated using the manufacturer's integrated software evaluation based on the equations of Ghoos *et al*. (1993). For determination of the recovery of ^{13}C tracer in breath over time, each participant's own physiologic production of CO_2 was taken into account and assumed to be 300 mmol per m^2 body surface area per hour (Braden, 2009). Body surface area was calculated according to the height-weight equation of Haycock, Schwartz & Wisotsky (1978) defined as body surface area (m^2) = (W0.5378 x H0.3964) x 0.024265, where W is participant's BM (kg) and H is participant's height (cm).

2.4. APPETITE ASSESSMENT

Appetite was assessed using 100 mm visual analogue scales (VAS). Ratings of hunger, fullness, prospective food consumption (Flint, Raben, Blundell & Astrup, 2000) as well as ratings of bloatedness and nausea were collected at baseline and at 10 min intervals following drink ingestion for 60 min. The VAS was composed of questions asking "how hungry do you feel," "how full do you feel," "how much do you think you can eat," "how bloated do you feel," and "how nauseous do you feel?" Respectively, horizontal lines 100 mm in length were anchored with "I am not hungry at all- I have never been more hungry", "not at all full- totally full", "nothing at all- a lot", "not at all bloated- very bloated" and "not at all nauseous- very nauseous" (Flint *et al*., 2000).

2.5. BLOOD SAMPLE PREPARATION AND ANALYSIS

Blood samples were collected in the studies reported in chapters 3 and 5 of this thesis by syringe into serum separator vacutainers (Becton Dickinson, Plymouth, UK) following withdrawal of 4-5 mL of blood to clear the catheter extension and 50 µl of DPP-IV inhibitor (Merck Millipore Limited, UK) and 50 µl of Pefabloc (Roche Diagnostics Limited, UK) immediately added to prevent the degradation of active GLP-1 by DPP-IV and acylated ghrelin by protease (10 µl/mL of whole blood of both inhibitors as recommended by the manufacturers). Samples were then kept on ice until centrifugation. All blood samples were collected with the participant in a semi-supine position and cannulas were kept patent by flushing with nonheparinized saline (0.9% sodium chloride; Becton Dickinson, New Jersey, USA). Blood samples were centrifuged (Z400K, Hermle, Germany) at 1500 **g** for 15 min at 4ºC. One serum aliquot was stored at 4ºC for later analysis for osmolality and the rest immediately stored at -80ºC for later biochemical analysis.

Serum glucose concentration were determined using the glucose oxidase phenol 4-aminoantipyrine peroxidase (GOD-PAP) method on a clinical chemistry analyser (Daytona; Randox Laboratories Ltd, UK) and serum fructose concentration were determined using a colorimetric assay (EnzyChrom^{TM} EFRU-100; BioAssay Systems, CA, USA). Mean intra-assay coefficient of variation (CV) for glucose was 1.3% and for fructose 5.1%. Concentrations of serum L-lactate and triglycerides, were also determined on the clinical chemistry analyser to assess hepatic metabolism of fructose. Mean intra-assay CVs were 7.0% and 6.0%, respectively. Concentrations of insulin, active GLP-1, total GIP, and acylated ghrelin were determined using a human gut hormone multiplex assay (Milliplex MAP, Merck Millipore Ltd, UK). Mean intra-assay CVs were 8.1%, 20.8%, 4.8% and 5.5%, and mean inter-assay CVs were 5.7%, 9.6%, 8.7% and 17.3%, respectively.

2.6. OTHER ANALYSIS

All test drink and urine samples were stored at 4 ºC until analysis of osmolality by freezing point depression (Gonotec Osmomat 030 Cryoscopic Osmometer; Gonotec, Berlin, Germany).

2.7. PARTICIPANT CRITERIA AND ETHICAL APPROVAL

All participants were non-smokers, had no history of gastrointestinal symptoms or disease, were not taking any medication with any known effect on gastrointestinal function and had no other medical conditions as assessed by a medical screening questionnaire. Verbal and

written explanations of the experimental procedures were given prior to participation and all participants provided written informed consent. All studies were conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures were granted approval by the Ethical Advisory Committee of Manchester Metropolitan University's Faculty of Science and Engineering (Appendix 1).

3. THE EFFECT OF DIFFERENT SIMPLE SUGARS ON GASTRIC EMPTYING RATE, GASTROINTESTINAL HORMONES, AND HEPATIC METABOLISM AND FUNCTION[1]

[1]Some of the data from this study contained within this chapter was presented as a poster communication and the abstract published in "Yau, A., McLaughlin, J., Maughan, R.J., Gilmore, W. & Evans, G.H. (2013). The influence of simple sugars on gut hormone response and gastric emptying rate. *International Journal of Sport Nutrition and Exercise Metabolism*, 23, S13."

3.1. INTRODUCTION

High quantities of the monosaccharide fructose are found naturally in foods and beverages (e.g. fruits), but its broadened use as an added ingredient, either as sucrose or high fructose corn syrup (mixture of fructose and glucose typically at 55:45 ratio) (HFCS), in soft drinks and other sweetened beverages has greatly increased its dietary consumption (Johnson & Murray 2010; Lindqvist, Baelemans & Erlanson-Albertsson, 2008). Excessive intake of fructose and over-consumption of sugary beverages have been suggested to contribute to the development of the metabolic syndrome and the epidemic-like increase in obesity through altering feeding patterns and the promotion of weight gain (Lindqvist *et al*., 2008). The physiological mechanism as to how this may occur, however, is incomplete and whether certain sugars are more harmful than others still requires much elucidation. The effect of these different carbohydrate types on gastric emptying (the rate at which food/drink is emptied from the stomach), and the response of various appetite hormones are an important consideration.

Research comparing the rate of gastric emptying between solutions of the common sugars glucose (monosaccharide), fructose (monosaccharide), and sucrose (disaccharide of glucose and fructose) are few and equivocal. Several studies have reported that fructose empties faster from the stomach than does glucose at the same concentration (Horowitz, Cunningham, Wishart, Jones & Read, 1996; Sole & Noakes, 1989; Moran & McHugh, 1981; Elias, Gibson, Greenwood, Hunt & Tripp, 1968), whereas others have found no differences (McGlone, Little & Thompson, 2008; Shi, Bartoli, Horn & Murray, 2000). Fructose (Elias *et al*., 1968) and glucose (Murray, Eddy, Bartoli & Paul, 1994) have also independently been reported to empty faster and slower than sucrose, respectively. Similarly, a combined fructose and glucose solution has been reported to empty faster than an equimolar glucose only solution (Jeukendrup & Moseley, 2010), but others reported no difference (Shi *et al*., 2000).

A small compilation of parallel research investigating the effects of these different oral carbohydrate solutions on various peripheral hormones that influence appetite also exists. Previous research has shown fructose stimulates GLP-1^{7-36} (Kong, Chapman, Goble, Wishart, Wittert, Morris *et al*., 1999), insulin (Bray, 2010; Teff, Grudziak, Townsend, Dunn, Grant, Adams *et al*., 2009; Bowen, Noakes & Clifton, 2007; Kong *et al*., 1999) and leptin (Bray, 2010) secretion, and suppresses ghrelin (Teff, Elliott, Tschop, Kieffer, Radar, Heiman *et al*., 2004) to a lesser degree than comparable amounts of glucose. On the other hand, others have also shown no differences in GLP-1^{7-36} and ghrelin responses between ingestion of glucose and of fructose (Bowen *et al*., 2007) and no difference in plasma

insulin, leptin and ghrelin levels following oral intakes of similar carbohydrates HFCS (55% fructose: 45% glucose) and sucrose (Melanson, Zukley, Lowndes, Nguyen, Angelopoulos & Rippe, 2007). Furthermore, two weeks of glucose, sucrose and fructose consumption has also been shown to decrease circulating levels of PYY, with the latter monosaccharide also increasing fasting ghrelin and insulin within the same time-frame in rats (Lindqvist *et al*., 2008). No study has concurrently investigated or compared the gastric emptying of all three of these sugars plus a glucose-fructose mixture, while also studying at the same time the circulatory responses of the most important panel of appetite hormones promoted by carbohydrate ingestion in humans. Moreover, the human studies aforementioned have all involved the ingestion of large and untypical amounts of glucose and fructose ranging from 50 g to approximately 135 g (30% of estimated energy requirements). In addition, studies that have measured ghrelin have consistently examined total ghrelin and not the active form acylated ghrelin (Lindqvist, *et al*., 2008; Bowen *et al*., 2007; Teff *et al*., 2004).

The consumption of fructose is also progressively being linked with non-alcoholic fatty liver disease (NAFLD) through its unfavourable hepatic metabolism (Vos & Lavine, 2013; Tappy & Le, 2012; Yilmaz, 2012). NAFLD is an increasingly prevalent chronic liver disease that is characterised by elevated intrahepatic fat and mitochondrial dysfunction (Ferder, Ferder & Inserra, 2010). As mentioned in chapter one, high fructose ingestion is considered to favour lipogenesis and triglyceride synthesis by serving as a relatively unregulated source of acetyl coA and glycerol-3-phosphate for hepatic lipogenesis (Bray, Nielsen & Popkin, 2004; Elliott *et al*., 2002). There is strong evidence in humans that short to moderate-term overfeeding with large amounts of fructose results in larger increases in fasting and postprandial plasma triglyceride concentrations than glucose (Stanhope, Bremer, Medici, Nakajima, Ito, Nakano *et al*., 2011; Ngo Sock, Le, Ith, Kreis, Boesch & Tappy, 2010; Stanhope, Schwarz, Keim, Griffen, Bremer, Graham *et al*., 2009; Teff *et al*., 2009; Stanhope, Griffen, Bair, Swarbrick, Keim & Havel, 2008; Teff *et al*., 2004; Bantle, Raatz, Thomas & Gerogopoulos, 2000). There is also evidence to show short to moderate-term overfeeding with fructose suppresses non-esterified fatty acid (NEFA) (Le, Faeh, Stettler, Ith, Kreis, Vennathen, *et al*., 2006; Teff *et al*., 2004), and ß-hydroxybutyrate (Ngo Sock *et al*., 2010; Le *et al*., 2006) concentrations, indicating decreased lipolysis and metabolism of NEFA, respectively. In some cases, however, the effects of fructose on these measures have been shown to be comparable to glucose (Ngo Sock *et al*., 2010; Teff *et al*., 2009; Teff *et al*., 2004) or have not been compared at all (Le *et al*., 2006). In addition, a marker of liver damage such as circulating alanine aminotransferase (ALT) has been

reported in a recent meta-analysis to increase following excess intake of fructose but was no different in comparison to glucose (Chiu, Sievenpiper, de Souza, Cozma, Mirrahimi, Carleton *et al*., 2014).

There is, however, comparatively little data on the effect of a single acute bolus of different carbohydrates on markers of hepatic metabolism and function. The aforementioned studies have investigated the effects of increased fructose consumption for 1 day to 10 weeks. One recent study that has compared the effect of a single mixed glucose and fructose solution (45:55 g), to mimic HFCS, reported lactate and NEFA responses were significantly greater than that elicited by 100 g of glucose alone (Bidwell, Homstrup, Doyle & Fairchild, 2010). The authors found no difference in triglyceride response, however. On the other hand, another study by Parks, Skokan, Timlin & Dingfelder (2008) showed acute ingestion of either a 50:50 or a 25:75 solution containing 42.7 g glucose: 42.7 g fructose and 21.3 g glucose: 64.1 g fructose, respectively, resulted in significantly greater serum triglyceride concentrations than 85 g glucose alone. Studies that have compared the acute effects of HFCS and sucrose have unsurprisingly found similar responses as they contain very similar amounts of glucose and fructose. Consumption of 68 g HFCS (39 g fructose and 29 g glucose) (Le, Frye, Rivard, Cheng, McFann, Segal *et al*., 2012) and at equivalent proportions for 25% of energy intake (Stanhope *et al*., 2008) showed no difference in postprandial triglyceride levels compared to intakes of matched amounts of sucrose. No difference in lactate response was also reported (Le *et al*., 2012).

Moreover, all of the dietary intervention and acute ingestion studies investigating the effects of fructose on hepatic metabolism and function have involved feeding of very high doses of sugars or fructose ranging from acute boluses of 68 g or above to approximately 188 g per day (30% energy requirements of guideline daily amount for average men). The effect of a much smaller amount reflective of a typical serving is unknown.

Therefore, the aims of this study were to examine the effect of different isoenergetic oral carbohydrate solutions on:

1) Gastric emptying rate,
2) Circulating gut hormone responses,
3) Hepatic metabolic responses and function, and
4) Subjective feelings of appetite and satiety.

3.2. METHODS

3.2.1. Participants

Seven healthy male volunteers (mean ± S.D, age 25 ± 4 y, height 179 ± 8 cm, BM 82 ± 12 kg, BMI 26 ± 4 $kg.m^{-2}$, and estimated body fat percentage 21 ± 7%) participated in the present investigation.

3.2.2. Experimental trials

Participants reported to the laboratory on five occasions, each separated by a minimum of 6 d. In addition to the pre-trial conditions outlined in general methods, participants were also asked to record their food and drink intake as well as physical activity during the 24 h prior to their first experimental visit and asked to replicate these for their subsequent visits.

Upon arrival at the laboratory, participants were asked to completely empty their bladder into a container from which a 5 mL urine sample was retained for later analysis of osmolality. Body mass was subsequently recorded. Following this a 21 gauge intravenous cannula (Venflon; Becton Dickinson, Plymouth, UK) was inserted into an antecubital vein and a catheter extension (Vygon, Ecouen, France) attached. A baseline blood sample was then obtained using the procedure outlined in general methods. Participants then ingested 595 mL of one of the following test drink solutions within two min; water (W), 6% glucose (GLU), 6% fructose (FRU), 6% sucrose (SUC) or 6% combined glucose and fructose (50:50; GLU+FRU). All carbohydrate solutions were equicaloric, respectively consisting of 39.6 g glucose monohydrate, 36 g fructose, 36 g sucrose, and 19.8 g glucose monohydrate + 18 g fructose, dissolved in commercially available natural mineral water (Evian, Danone Ltd, France) to a volume of 600 mL. Glucose and fructose were purchased from MyProtein (www.myprotein.com) and sucrose from a local supermarket (Granulated Sugar; Silverspoon, UK). Participants were given a maximum of two min to consume the test solution and instructed to consume it as quickly as they were able to. Drink solutions were given at room temperature and a 5 mL sample of the drink was retained for later analysis of osmolality. Participants remained in a semi-supine position throughout the drink ingestion and 60 min sampling procedure. Further blood samples were obtained at 10, 20, 30 and 60 min post-drink ingestion and gastric emptying rate and appetite was assessed for the duration of study as described in general methods. Following all sample collections at 60 min, the cannula was removed and participants were asked again to completely empty their bladder into a container and a 5 mL urine sample was again retained for later analysis.

3.2.3. Biochemical analysis

In addition to the blood sample analysis described in general methods, ALT, NEFA and D-3 Hydroxybutyrate (Ranbut) were also determined on the clinical chemistry analyser to assess hepatic metabolism and function. The NEFA assay was performed separately from the other analytes due to interference with the triglyceride assay. Gut hormone analysis was performed in single analysis with the exception of 16 randomly selected samples which were performed in duplicate. Corresponding intra-assay coefficient of variations (CV) for ghrelin, GIP, GLP-1 and insulin were 6%, 4.8%, 24% and 11.8%, respectively. Inter-assay CVs for ghrelin, GIP, GLP-1 and insulin were 17.9%, 4.2%, 5.1% and 3.8%, respectively. Lactate, triglyceride, ALT, D-3 Hydroxybutyrate, and NEFA analysis was performed in duplicate and intra-assay CVs 12.5%, 10.4%, 9.5%, 4.9% and 3.8%, respectively. Glucose analysis was performed in single analysis as well as fructose, where the CV of a preliminary assay was 2.5%.

3.2.4. Statistical analysis

Area under curve for gastric emptying DOB and gut hormone data were calculated using polynomial curves of best fit and mathematical integration, and the trapezoid method, respectively. Differences in pre-trial BM, pre-trial urine osmolality, drink osmolality, gastric emptying $T_{½}$ and T_{lag}, gastric emptying DOB AUC, and gut hormone concentration AUC were examined using one-way repeated analysis of variance (ANOVA). Significant *F*-tests were followed by Bonferroni adjusted pairwise comparisons. Two-way repeated ANOVA were used to examine differences in gastric emptying DOB values, urine osmolality, serum osmolality, blood glucose and fructose concentrations, gut hormone concentrations, and subjective appetite VAS scores. Significant *F*-tests were followed with the appropriate paired Student's *t*-tests or one-way repeated ANOVA and Bonferroni adjusted pairwise comparisons. Sphericity for repeated measures was assessed, and where appropriate, Greenhouse-Geisser corrections were applied for epsilon < 0.75, and the Huynh-Feldt correction adopted for less severe asphericity. All data were analysed using SPSS Statistics for Windows version 19 (IBM, New York, US). Statistical significance was accepted at the 5% level and results presented as means ± standard deviation (SD).

3.3. RESULTS

3.3.1. Body mass and hydration status

Body mass was stable over the duration of the study. Hydration status based on urine osmolality and serum osmolality was also consistent prior to each trial. Data are presented

in Table 2. Urine output 60 min post-drink ingestion was not different between trials (W, 613 ± 268 mL; FRU, 411 ± 254 mL; GLU, 639 ± 226 mL; SUC, 577 ± 400 mL; GLU+FRU, 596 ± 331 mL; P = 0.231). Two-way ANOVA and post hoc analyses revealed urine osmolality significantly decreased by 300 ± 201 mOsmol/kg, 209 ± 174 mOsmol/kg, 239 ± 197 mOsmol/kg, 399 ± 209 mOsmol/kg post-trial for W ($P < 0.01$), FRU ($P < 0.05$), GLU ($P < 0.05$) and SUC ($P < 0.01$) respectively. However, there was no significant decrease for GLU+FRU (170 ± 298 mOsmol/kg; P = 0.221).

Table 2. Pre-trial body mass and hydration markers (n 7).

	W	FRU	GLU	SUC	GLU+FRU	*P*-value
Body mass (kg)	81.52 ± 12.03	81.80 ± 12.31	81.84 ± 11.77	81.93 ± 12.06	81.54 ± 12.42	0.638
Urine osmolality (mOsmol/kg)	461 ± 232	431 ± 174	375 ± 224	593 ± 309	465 ± 260	0.504
Serum osmolality (mOsmol/kg)	292 ± 4	293 ± 4	292 ± 1	291 ± 4	292 ± 3	0.729

Two-way ANOVA for serum osmolality revealed no main effect of trial (P = 0.271), no main effect of time (P = 0.358), but a significant interaction effect ($P < 0.05$). Post hoc analyses indicated a significant decrease over time for W ($P < 0.05$), though the location of this difference could not be determined. A significant difference between trials at 30 min was also indicated ($P < 0.05$) but again the difference could not be located.

3.3.2. Drink osmolality

Osmolality of the drink solutions were 13 ± 1 mOsmol/kg, 368 ± 4 mOsmol/kg, 370 ± 6 mOsmol/kg, 204 ± 1 mOsmol/kg and 369 ± 4 mOsmol/kg for W, FRU, GLU, SUC and GLU+FRU, respectively. Mean water osmolality was significantly lower than all other solutions ($P < 0.001$) and SUC was significantly lower than GLU, FRU, and GLU+FRU ($P < 0.001$).

3.3.3. Gastric emptying

No significant differences were found between trials for gastric emptying $T_{½}$ (P = 0.136). There was, however, considerable inter-individual and between-trial variation in $T_{½}$. One-way ANOVA and post hoc analyses revealed T_{lag} was significantly greater for GLU when compared with SUC (51 ± 26 min vs. 25 ± 18 min; $P < 0.05$) (Figure 2). Two-way ANOVA

for delta over baseline (DOB) data showed no main effect of trial (P = 0.250), but a significant main effect of time (P < 0.001), and a significant interaction effect (P < 0.01). Post hoc analyses revealed a significantly greater DOB for SUC compared to GLU at 10 min post drink ingestion (P < 0.05) (Figure 3a). No statistical differences in mean AUC over the 60 min post drink ingestion were demonstrated (P = 0.209) (Figure 3b). Gastric emptying DOB results are also expressed as a percentage of maximum (Figure 4). Two-way ANOVA showed no main effect of trial (P = 0.224), a significant main effect of time (P < 0.001) and significant interaction effect (P < 0.001). Post hoc analysis revealed percentage of maximum DOB for SUC was significantly higher than W (P < 0.01), FRU (P < 0.05) and GLU (P < 0.05) at 10 min. Furthermore, at 50 min, GLU was significantly greater than W (P < 0.05) and at 60 min GLU was significantly greater than W (P < 0.01), SUC (P < 0.05) and GLU+FRU (P < 0.05).

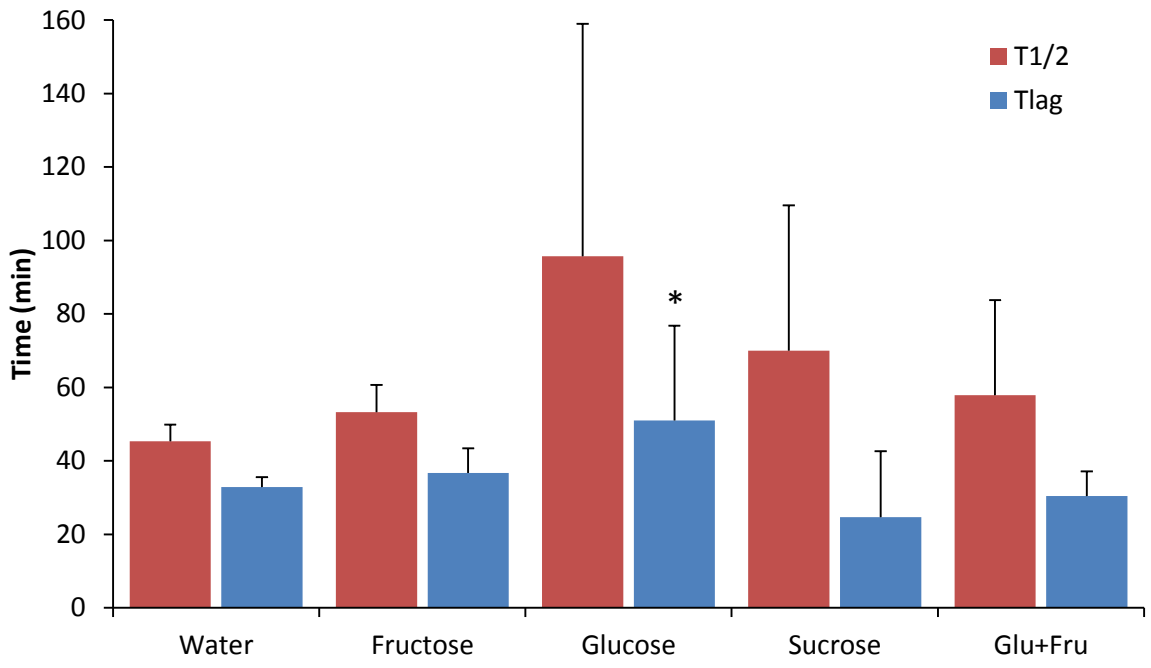

Figure 2. Gastric emptying $T_{½}$ and T_{lag} for ingestion of 595 mL water, 6% fructose, 6% glucose, 6% sucrose and 6% combined glucose and fructose solutions. *Significantly higher than sucrose (P < 0.01). Values are mean ± SD (n 7).

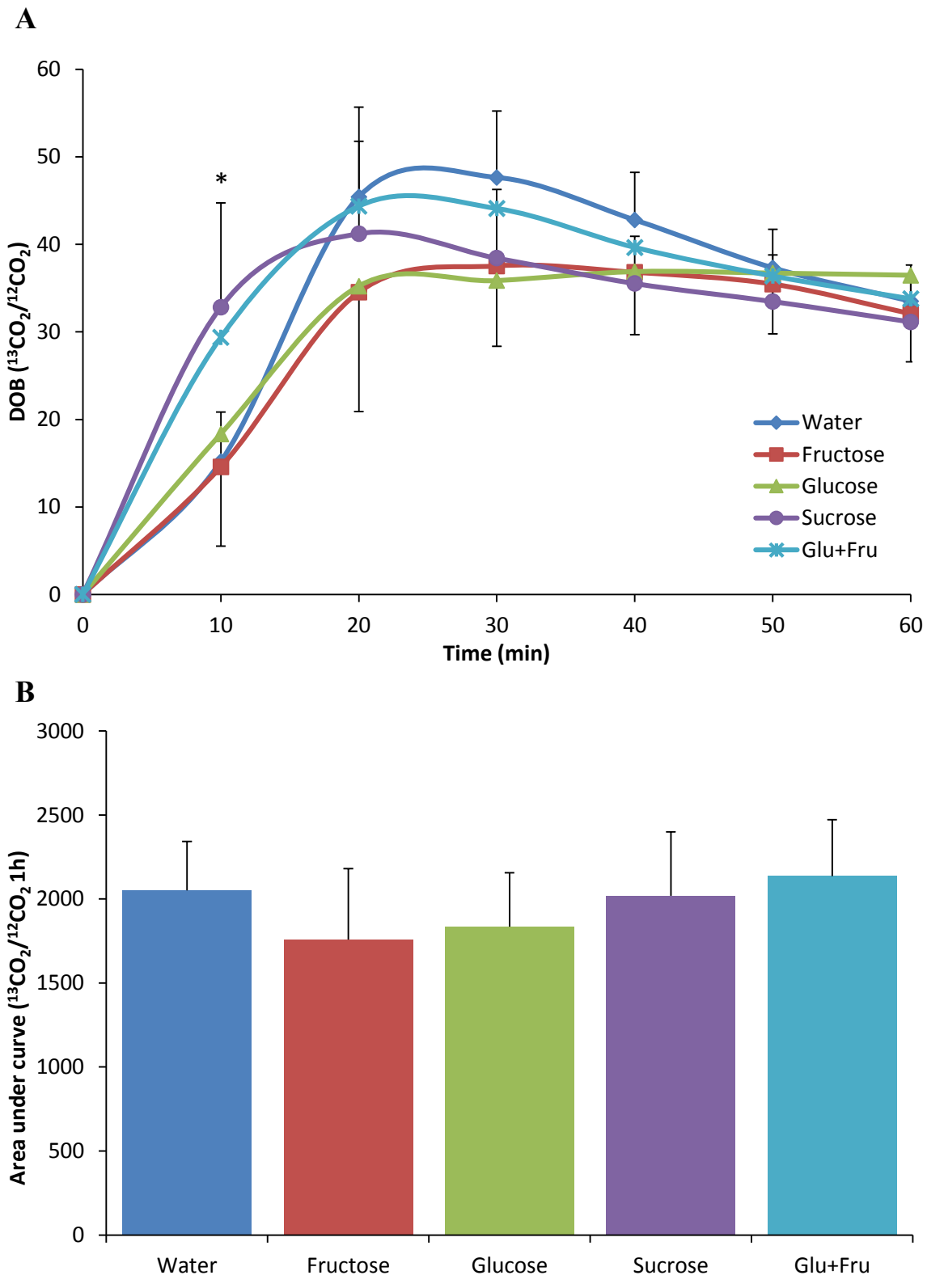

Figure 3. Gastric emptying. (A) Delta over baseline over (DOB) 60 min post ingestion of 595 mL water, 6% fructose, 6% glucose, 6% sucrose and 6% combined glucose and fructose solutions. (B) Area under curve. *Sucrose significantly higher than glucose ($P <$ 0.05). Values are mean ± SD (n 7).

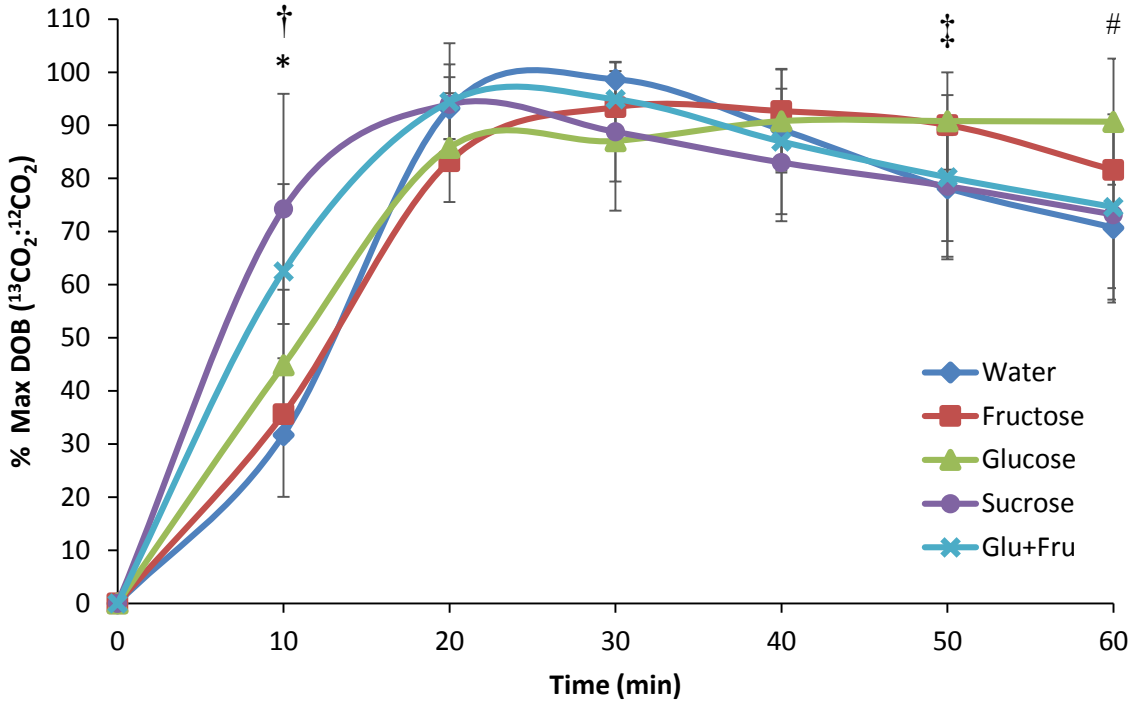

Figure 4. Gastric emptying delta over baseline (DOB) normalised as percentage of maximum over 60 min post ingestion of 595 mL water, 6% fructose, 6% glucose, 6% sucrose and 6% combined glucose and fructose solutions. *Sucrose significantly higher than water, fructose and glucose ($P < 0.05$). †Combined significantly higher than water ($P < 0.05$). ‡Glucose significantly higher than water ($P < 0.05$). #Glucose significantly higher than water, sucrose and combined ($P < 0.05$). Values are mean ± SD (n 7).

3.3.4. Gut hormones

3.3.4.1. Ghrelin

Baseline ghrelin concentrations were not significantly different between trials (W, 232.08 ± 79.75 pg/mL; FRU, 189.04 ± 68.84 pg/mL; GLU, 200.95 ± 80.24 pg/mL; SUC, 220.69 ± 84.22 pg/mL; GLU+FRU, 189.89 ± 65.09 pg/mL; $P = 0.101$). Two-way ANOVA revealed significant effects of trial ($P < 0.05$), time ($P < 0.001$) and an interaction ($P < 0.01$). Concentrations changed significantly over time for all trials, with greater decreases following consumption of carbohydrate solutions compared with W (Figure 5a). Post hoc analyses indicated a significant difference between trials at 30 min and 60 min. The difference could not be located at 30 min whilst at 60 min ghrelin concentration for FRU was significantly lower than W (135.69 ± 61.79 pg/mL vs. 211.76 ± 90.39 pg/mL; $P < 0.05$). Area under curve values for ghrelin are presented in Figure 5b. A significant difference between ghrelin AUC existed with FRU being significantly smaller than W (9279.14 ± 3508.73 pg/mL 1h vs. 12032.71 ± 4278.31 pg/mL 1h; $P < 0.05$).

3.3.4.2. GIP

Baseline GIP concentrations were not significantly different between trials (W, 8.81 ± 3.33 pg/mL; FRU, 9.31 ± 5.26 pg/mL; GLU, 12.67 ± 7.71 pg/mL; SUC, 12.12 ± 8.82 pg/mL; GLU+FRU, 13.15 ± 7.20 pg/mL; $P = 0.266$). Two-way ANOVA revealed significant effects of trial ($P < 0.001$), time ($P < 0.001$) and interaction ($P < 0.001$). Concentrations increased significantly higher than baseline over time for GLU ($P < 0.001$), SUC ($P < 0.01$) and GLU+FRU ($P < 0.05$) but not for W ($P = 0.716$) or FRU ($P = 0.278$). Significant differences between GIP response occurred at 10, 20, 30 and 60 min post ingestion with the response being greatest for GLU (Figure 6a). Area under curve values for the trials were also significantly different ($P < 0.001$; Figure 6b).

3.3.4.3. GLP-1

There were no significant differences in baseline concentrations between trials (W, 3.59 ± 9.50 pg/mL; FRU, 3.59 ± 9.50 pg/mL; GLU, 9.31 ± 16.23 pg/mL; SUC, 13.14 ± 16.54 pg/mL; GLU+FRU, 24.53 ± 27.53 pg/mL; $P = 0.175$). Two-way ANOVA showed a strong trend to significance for trial ($P = 0.053$), a significant effect of time ($P < 0.05$) and an interaction effect ($P = 0.0003$). Post hoc analyses indicated GLP-1 concentration increased then significantly decreased from 30 min to 60 min for FRU ($P < 0.05$). A significant response was also indicated for GLU ($P < 0.05$) but differences in time points could not be located. Responses to SUC and GLU+FRU were tending to statistical significance ($P = 0.057$ and $P = 0.082$, respectively). Post hoc analysis between trials indicated a significant difference between trials at 10 min ($P < 0.05$) and 30 min ($P < 0.05$), though differences could not be pinpointed (Figure 7a). Area under curve values between trials were also tending to significance ($P = 0.064$; Figure 7b).

3.3.4.4. Insulin

Insulin concentrations at baseline were not significantly different between trials (W, 191.37 ± 88.46 pg/mL; FRU, 192.07 ± 102.33 pg/mL; GLU, 216.92 ± 163.10 pg/mL; SUC, 172.44 ± 103.44 pg/mL; GLU+FRU, 177.67 ± 89.36 pg/mL; $P = 0.493$). Two-way ANOVA showed significant effects of trial ($P < 0.05$), time ($P < 0.05$) and interaction ($P < 0.001$). Post hoc analyses indicated significant changes over time for all carbohydrate trials though differences in time points could only be located for GLU and SUC. Insulin concentrations significantly increased at 10 min from baseline for both GLU and SUC then decreased significantly for GLU only. Significant differences between trials at 10 ($P < 0.01$), 20 ($P < 0.05$) and 30 min ($P < 0.05$) as well as AUC ($P < 0.05$) were also indicated, but differences could not be identified (Figure 8a and 8b).

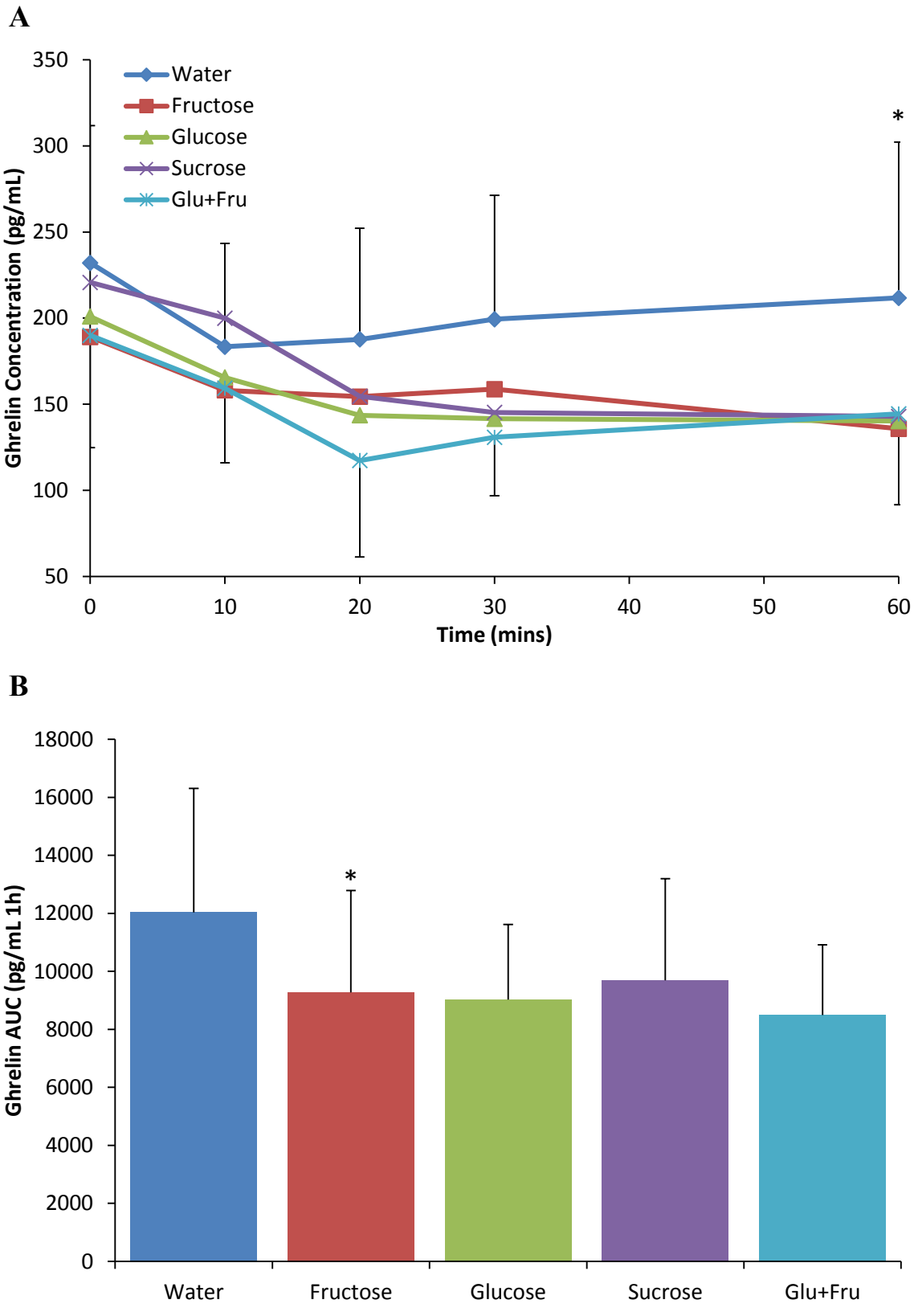

Figure 5. Gut hormone ghrelin (A) Response over 60 min post ingestion of 595 mL water, 6% fructose, 6% glucose, 6% sucrose and 6% combined glucose and fructose solutions. (B) Area under curve. *Fructose significantly lower than water ($P < 0.05$). Values are mean ± SD (n 7).

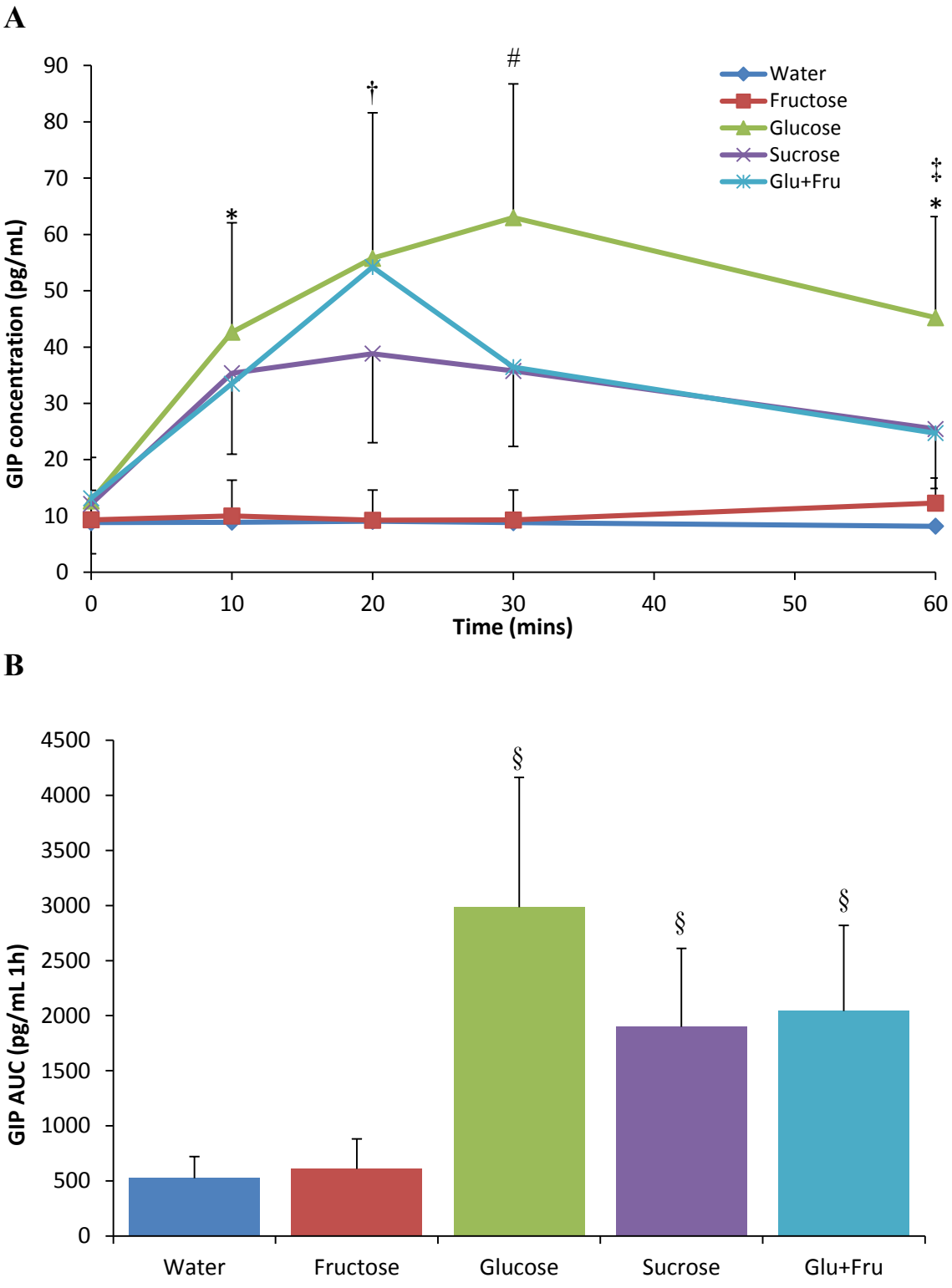

Figure 6. Gut hormone GIP. (A) Response over 60 min post ingestion of 595 mL water, 6% fructose, 6% glucose, 6% sucrose and 6% combined glucose and fructose solutions. *Glucose, sucrose and combined significantly higher than water ($P < 0.001$); †Glucose and sucrose significantly higher than water and fructose ($P < 0.01$); #Glucose, sucrose and combined significantly higher than water and fructose. Glucose also significantly higher than sucrose ($P < 0.001$); ‡Glucose significantly higher than fructose, sucrose and combined ($P < 0.01$). (B) Area under curve. §Significantly greater than water and fructose ($P < 0.001$). Values are mean ± SD (n 7).

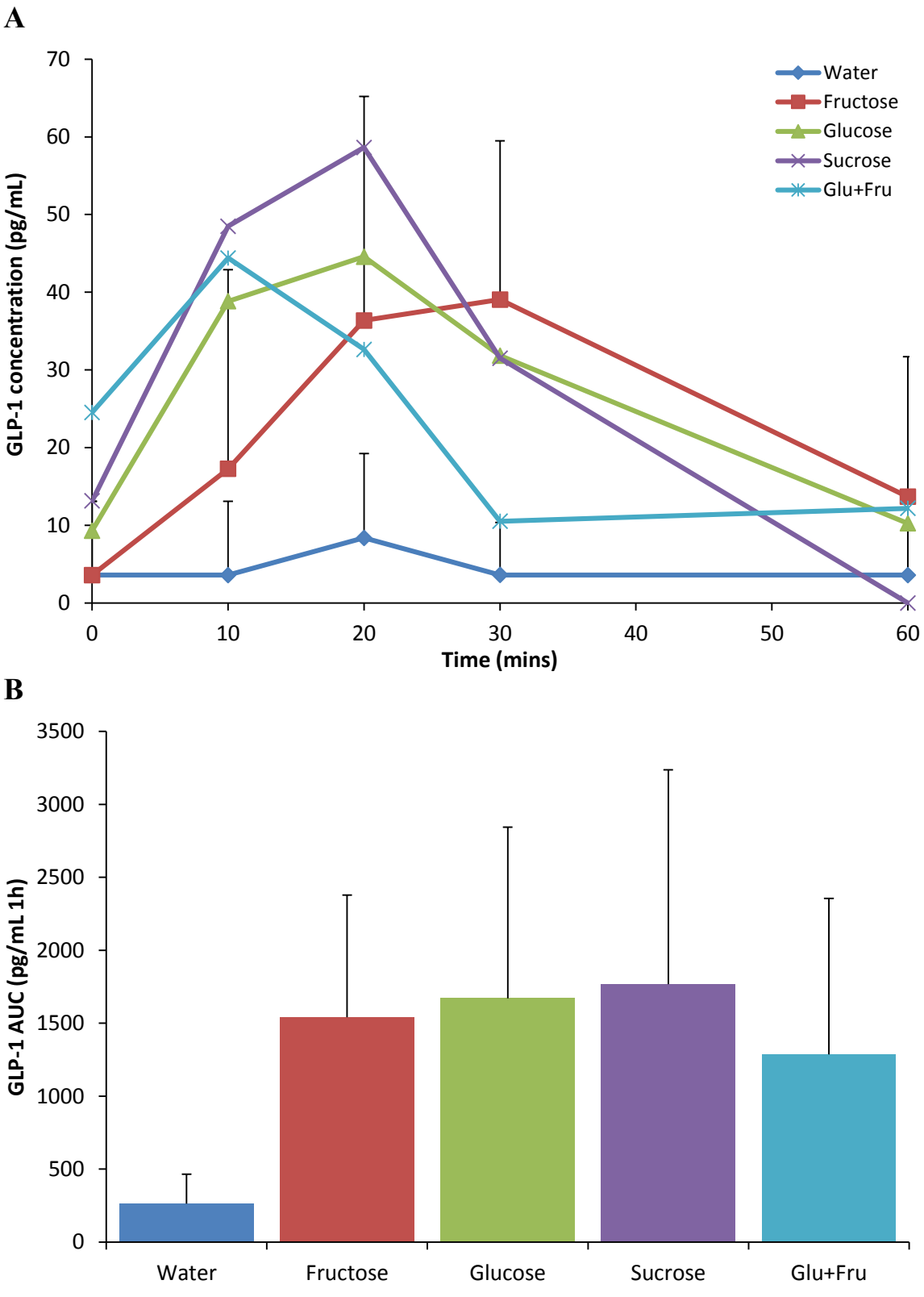

Figure 7. Gut hormone GLP-1 (A) Response over 60 min post ingestion of 595 mL water, 6% fructose, 6% glucose, 6% sucrose and 6% combined glucose and fructose solutions. (B) Area under curve. Values are mean ± SD (*n* 7).

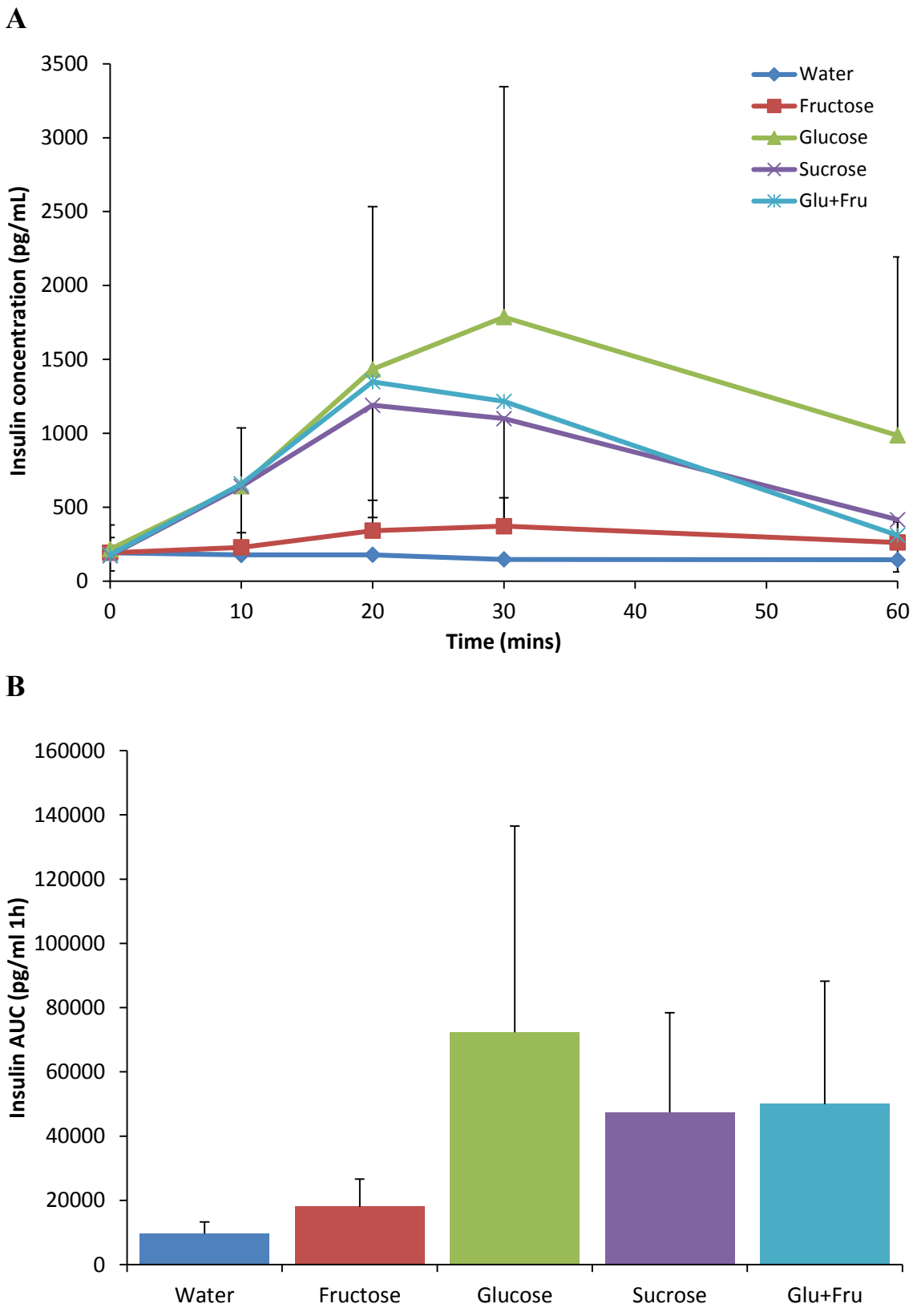

Figure 8. Hormone insulin (A) Response over 60 min post ingestion of 595 mL water, 6% fructose, 6% glucose, 6% sucrose and 6% combined glucose and fructose solutions. (B) Area under curve. Values are mean ± SD (*n* 7).

3.3.5. Blood glucose and fructose

Significant effects of trial ($P < 0.001$), time ($P < 0.001$) and interaction ($P < 0.001$) were seen for serum glucose concentration. Significant increases from baseline to 30 min post drink ingestion were followed by significant decreases at 60 min post ingestion for trials GLU ($P < 0.001$), SUC ($P < 0.001$) and GLU+FRU ($P < 0.001$). No change over time existed for W ($P = 0.323$) and no differences over time were located for FRU ($P = 0.007$). There were no differences in baseline serum glucose concentrations between trials ($P = 0.288$), nor at 60 min post ingestion ($P = 0.241$). However, differences in serum glucose response between trials were found at 10, 20 and 30 min ($P < 0.01$) (Figure 9a). These differences were also reflected in significant differences between AUC values (W, 301.44 ± 17.21 mmol/L 1h; FRU, 324.01 ± 9.84 mmol/L 1h; GLU, 403.24 ± 66.34 mmol/L 1h; SUC, 388.28 ± 18.13 mmol/L 1h; GLU+FRU, 373.73 ± 47.24 mmol/L 1h; $P < 0.001$) (Figure 9b).

Significant effects of trial ($P < 0.001$), time ($P < 0.01$) and interaction ($P < 0.001$) were also seen for serum fructose concentration. Fructose concentration significantly decreased at 30 min post drink ingestion compared to baseline for W, whilst in contrast, it significantly increased over time for FRU ($P < 0.001$), SUC ($P < 0.01$) and GLU+FRU ($P < 0.05$). No significant change occurred with GLU ($P = 0.234$). There were no differences in baseline serum fructose concentrations between trials ($P = 0.828$). Differences in response between trials existed at 10, 20, 30 and 60 min ($P < 0.001$) (Figure 10a). Mean AUC were significantly different with 2387.65 ± 1556.989 µmol/L 1h, 18885.35 ± 5798.358 µmol/L 1h, 3221.957 ± 2188.823 µmol/L 1h, 13352.59 ± 5931.096 µmol/L 1h and 12019.24 ± 5010.242 µmol/L 1h for W, FRU, GLU, SUC and GLU+FRU, respectively ($P < 0.001$) (Figure 10b).

3.3.6. Appetite and satiety

Hunger and prospective food consumption ratings tended to decrease at 10 min post drink ingestion and then generally recovered or increased thereafter for all trials. Fullness rating tended to increase or was unchanged 10 min following drink ingestion and then generally decreased thereafter. No significant differences were found for hunger (trial $P = 0.337$, time $P = 0.091$, interaction $P = 0.492$) or fullness (trial $P = 0.455$, time $P = 0.106$, interaction $P = 0.288$). A main effect of trial ($P = 0.652$) and interaction effect ($P = 0.430$) were also not present for prospective food consumption. A significant main effect of time ($P < 0.05$) was present, however. Post hoc analyses indicated a significant difference over time for GLU ($P < 0.05$), but differences in time-points could not be further located.

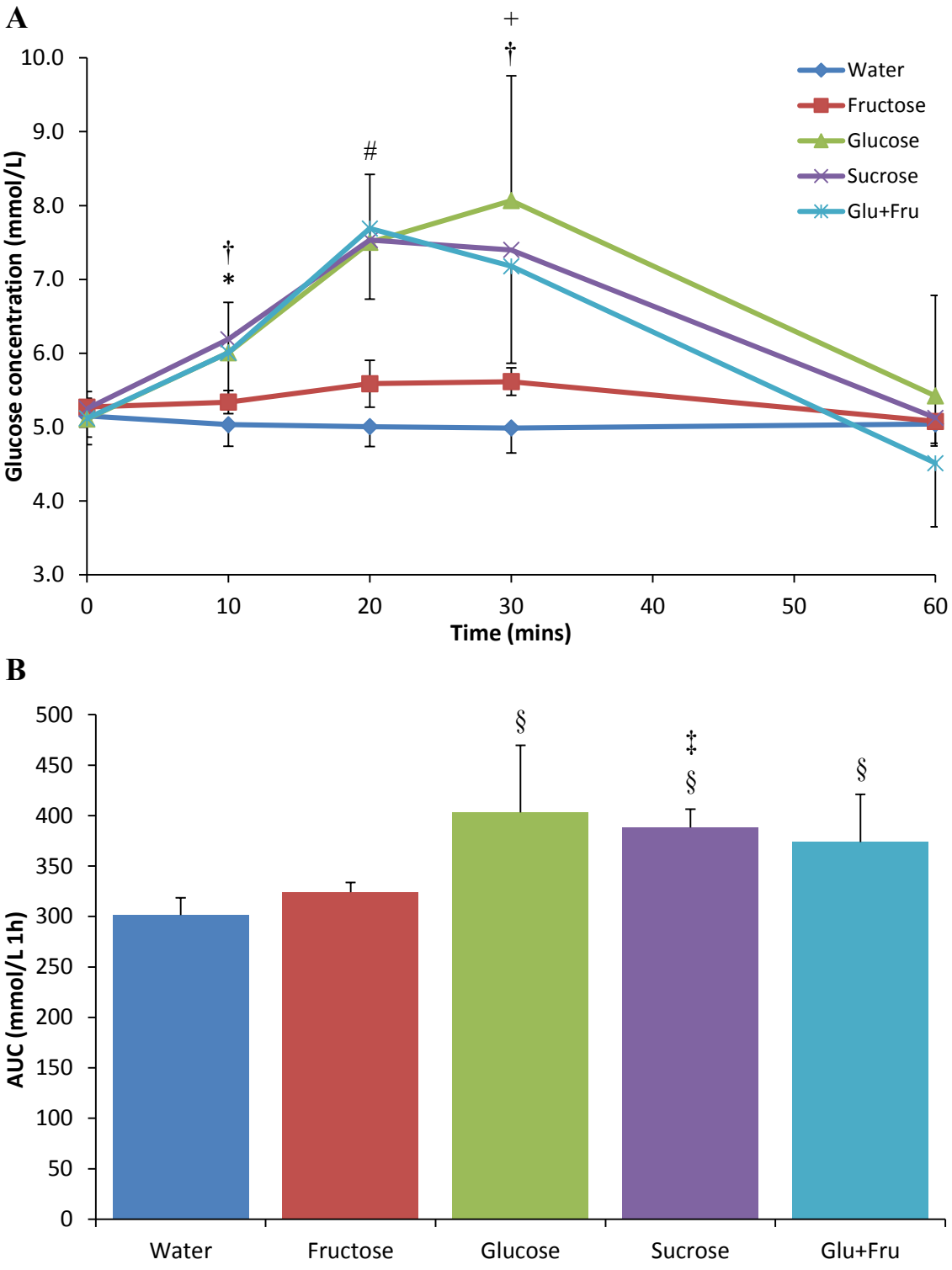

Figure 9. Serum glucose concentration. (A) Response over 60 min post ingestion of 595 mL water, 6% fructose, 6% glucose, 6% sucrose and 6% combined glucose and fructose solutions. *Glucose, sucrose and combined significantly higher than water ($P < 0.05$); †Sucrose significantly higher than fructose ($P < 0.05$); #Glucose, sucrose and combined significantly higher than water and fructose ($P < 0.05$); +All carbohydrate trials significantly higher than water ($P < 0.05$). (B) Area under curve. §Significantly greater than water; ‡Significantly greater than fructose ($P < 0.01$). Values are mean ± SD (n 7).

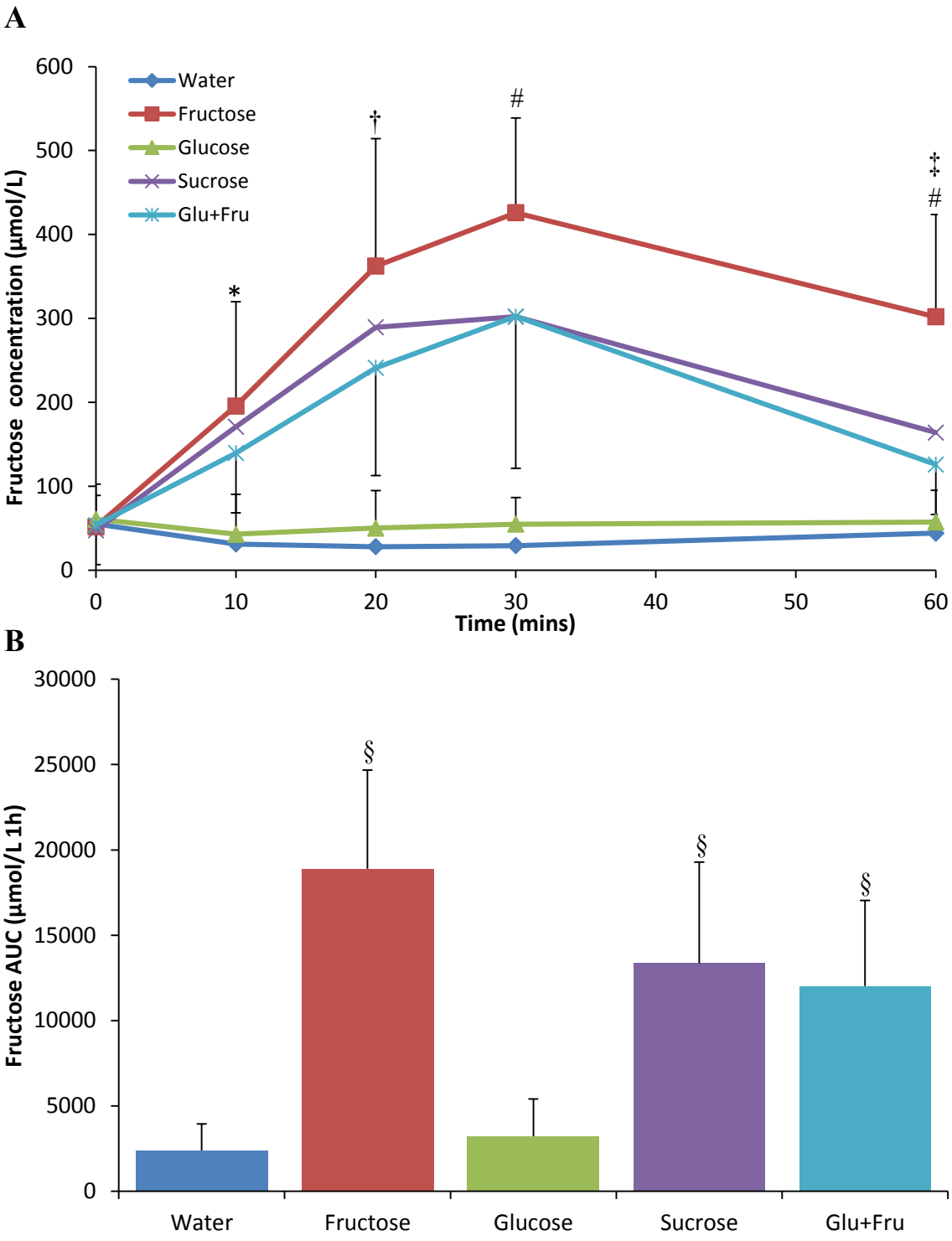

Figure 10. Serum fructose concentration. (A) Response over 60 min post ingestion of 595 mL water, 6% fructose, 6% glucose, 6% sucrose and 6% combined glucose and fructose solutions. *Sucrose significantly higher than water and glucose ($P < 0.01$); †Fructose significantly higher than water and glucose, combined significantly higher than water ($P < 0.05$); #Fructose and sucrose significantly higher than water and glucose ($P < 0.001$); ‡Fructose significantly higher than combined ($P < 0.001$). (B) Area under curve. §Significantly greater than water and glucose ($P < 0.001$). Values are mean ± SD (n 7).

3.3.7. Hepatic metabolism

3.3.7.1. Lactate

Two-way repeated ANOVA revealed significant main effects of trial ($P < 0.001$), time ($P < 0.001$) and interaction ($P < 0.001$). Serum lactate significantly increased over time in all four carbohydrate trials, whilst no difference was seen for W ($P = 0.447$) (Figure 11a). Lactate concentration following GLU ingestion increased most slowly with concentrations only reaching significant increases by the end of the trial in comparison to baseline and 20 min values. For FRU, however, significant increases were seen earlier on with values at 20, 30 and 60 min being greater than baseline ($P < 0.01$) and 10 min ($P < 0.05$). The increase in lactate following SUC ingestion was even more rapid, with significant increases from baseline seen from 10 min onwards ($P < 0.05$). However, the increase in lactate in the GLU+FRU trial was slightly slower than SUC and became significantly higher than baseline at 30 min post ingestion and thereafter ($P < 0.05$). Differences between trials at time-points were also seen. At 20 min, lactate was significantly higher in the SUC trial compared with W ($P < 0.05$), GLU ($P < 0.05$) and FRU ($P < 0.05$). At 30 min, in both FRU and SUC trials, lactate levels were significantly higher than in W ($P < 0.01$) and GLU ($P < 0.01$). Furthermore, at 60 min, lactate levels in all four carbohydrate trials were significantly higher than in W (GLU, $P < 0.05$; FRU, $P < 0.001$; SUC, $P < 0.05$; GLU+FRU, $P < 0.05$) with concentration in the FRU trial being significantly higher than in the GLU trial ($P < 0.01$). AUC values were significantly greater in the FRU ($P < 0.01$) and SUC trials ($P < 0.05$) compared with W, and also FRU ($P < 0.01$) and SUC ($P < 0.01$) compared with GLU (Figure 11b).

3.3.7.2. Triglycerides

Triglyceride response was very limited over the one-hour test period of this study. Two-way repeated ANOVA showed no effect of trial ($P = 0.425$) and time ($P = 0.254$) but a significant interaction effect ($P = 0.032$). Post-hoc analysis showed no differences between trials at time-points although there was a trend of difference at 10 min ($P = 0.099$; Figure 12a). A significant difference over time for W trial was indicated ($P = 0.039$) though pairwise comparisons did not locate any differences. Trends over time were also seen for GLU ($P = 0.053$), SUC ($P = 0.092$) and GLU+FRU ($P = 0.073$) trials. Changes in triglyceride concentration for FRU were not significantly different ($P = 0.279$). No differences were observed with AUC values ($P = 0.439$; Figure 12b).

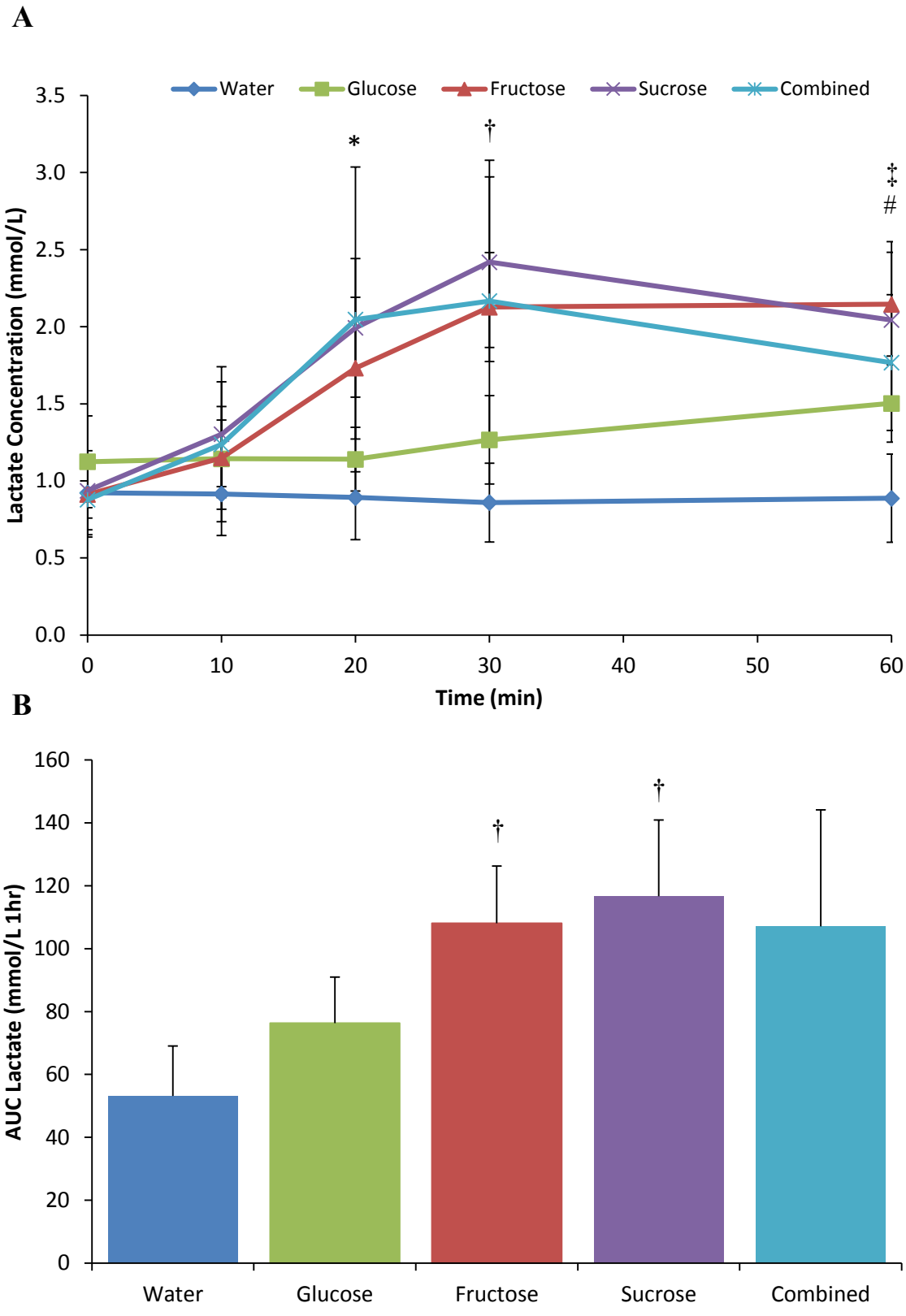

Figure 11. Serum lactate concentration. (A) Response over 60 min and (B) Area under curve post ingestion of 595 mL water, 6% fructose, 6% glucose, 6% sucrose and 6% combined glucose and fructose solutions.*Sucrose significantly greater than water, glucose and fructose ($P < 0.05$). †Fructose and sucrose significantly greater than water and glucose ($P < 0.01$). #All carbohydrate trials significantly greater than water ($P < 0.05$). ‡Fructose significantly greater than glucose ($P < 0.01$). Values are mean ± SD (n 7).

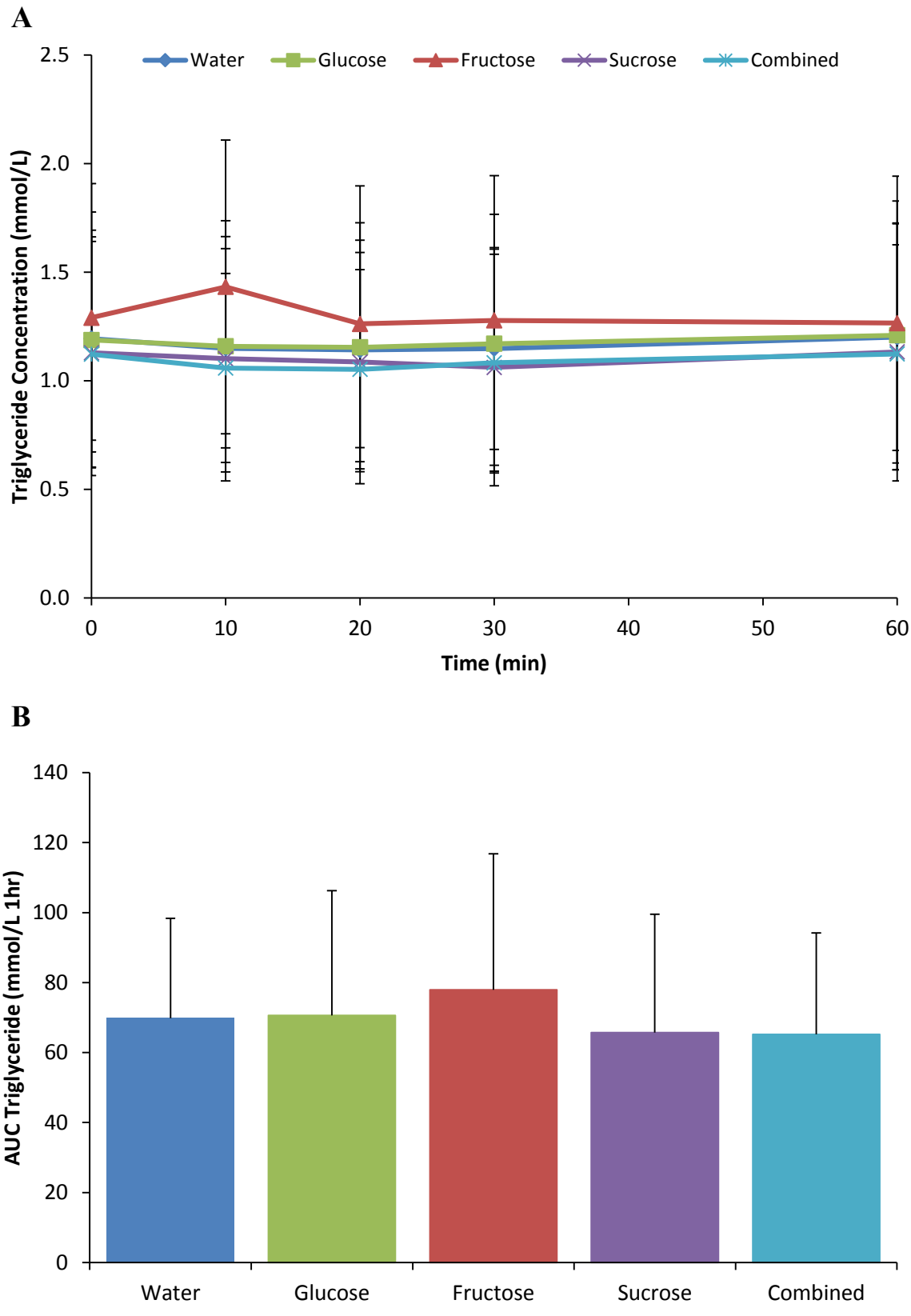

Figure 12. Serum triglyceride concentration. (A) Response over 60 min and (B) Area under curve post ingestion of 595 mL water, 6% fructose, 6% glucose, 6% sucrose and 6% combined glucose and fructose solutions. Values are mean ± SD (*n* 7).

3.3.7.3. D-3 Hydroxybutyrate (Ranbut)

No effect of trial (P = 0.220) or interaction (P = 0.891) and only a slight trend for an effect of time (P = 0.098) was seen for circulating D-3 hydroxybutyrate levels (Figure 13a). No differences between trials in AUC levels were seen (P = 0.179; Figure 13b).

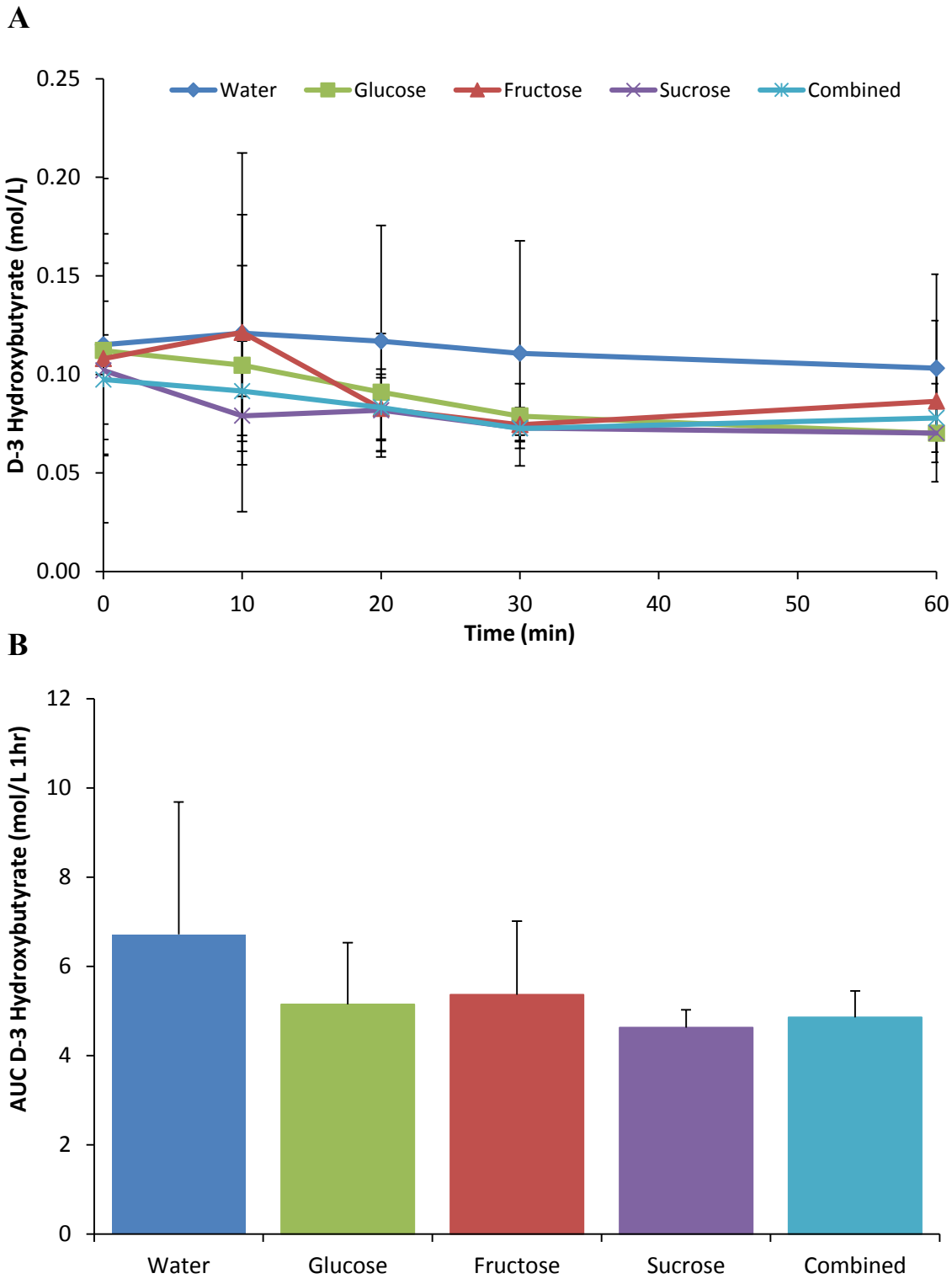

Figure 13. Serum D-3 hydroxybutyrate concentration. (A) Response over 60 min and (B) Area under curve post ingestion of 595 mL water, 6% fructose, 6% glucose, 6% sucrose and 6% combined glucose and fructose solutions. Values are mean ± SD (n 7).

3.3.7.4. ALT

As with D-3 hydroxybutyrate, serum ALT responses were not significantly different between trials but again there was a slight trend for an effect of time (trial $P = 0.493$, time $P = 0.084$, interaction $P = 0.504$; Figure 14a). No differences in AUC values were observed ($P = 0.510$; Figure 14b).

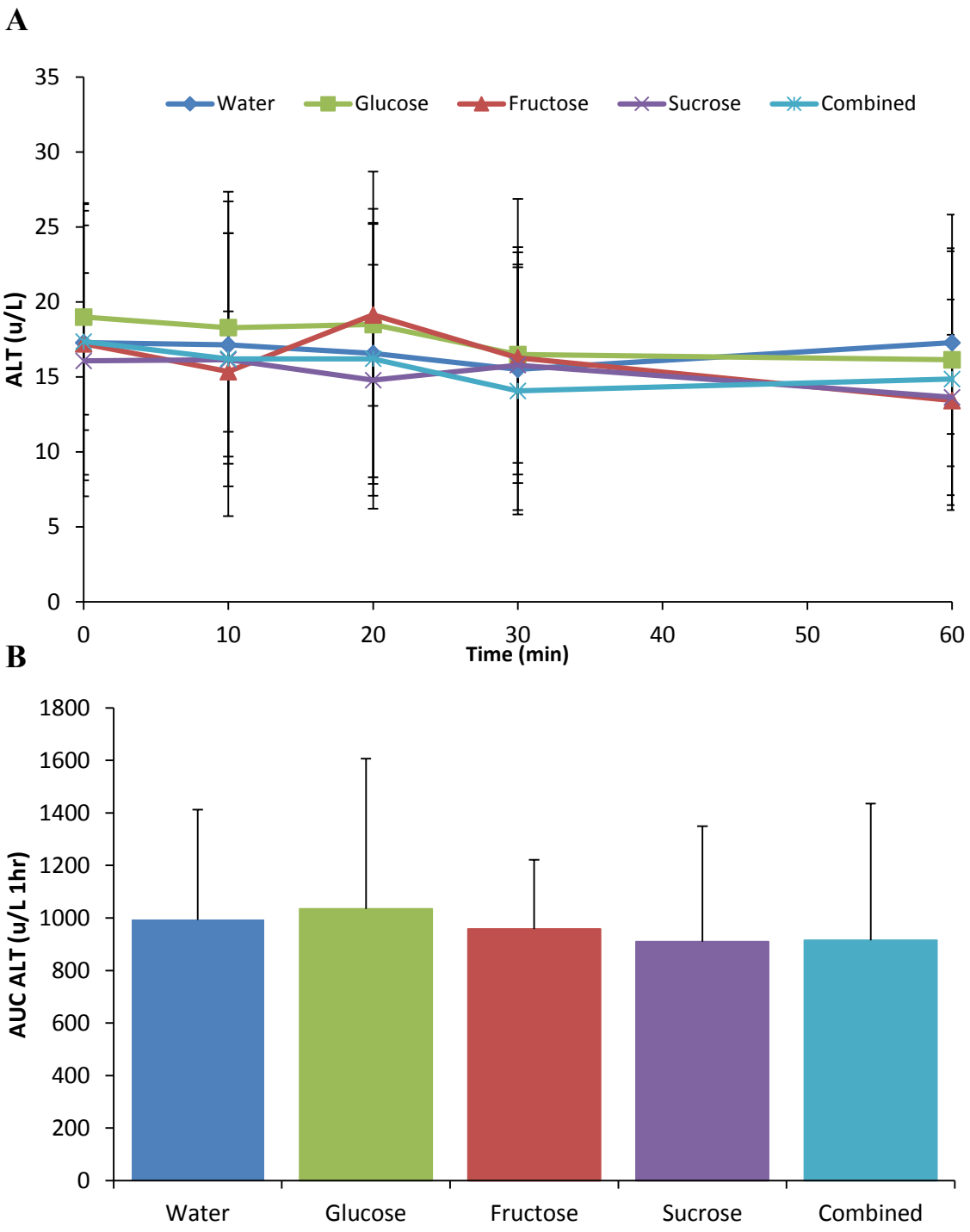

Figure 14. Serum alanine aminotransferase concentration (ALT). (A) Response over 60 min and (B) Area under curve post ingestion of 595 mL water, 6% fructose, 6% glucose, 6% sucrose and 6% combined glucose and fructose solutions. Values are mean ± SD (*n* 7).

3.3.7.5. NEFA

Two-way repeated ANOVA revealed no main effect of trial ($P = 0.411$), a significant effect of time ($P < 0.01$) and no interaction effect ($P = 0.431$) (Figure 15a). Post-hoc tests showed non-esterified fatty acid concentration decreased significantly over time for W ($P < 0.01$), GLU ($P < 0.001$) and GLU+FRU ($P < 0.001$) trials. For W, concentration at 20 and 30 min were significantly lower than baseline ($P < 0.05$). For GLU, concentrations from 20 min onwards were all significantly lower than both baseline and at 10 min ($P < 0.01$ and $P < 0.05$, respectively), and at 60 min concentrations were further lower than 30 min levels ($P < 0.05$). Similarly, for GLU+FRU trial, concentrations at 20, 30 and 60 min were all significantly lower than baseline ($P < 0.05$, $P < 0.001$ and $P < 0.01$, respectively). Levels at 30 and 60 min were also significantly lower than at 10 min ($P < 0.05$ and $P < 0.01$) and the level at 60 min lower than that at 20 min ($P < 0.01$). No differences in AUC were observed ($P = 0.370$; Figure 15b).

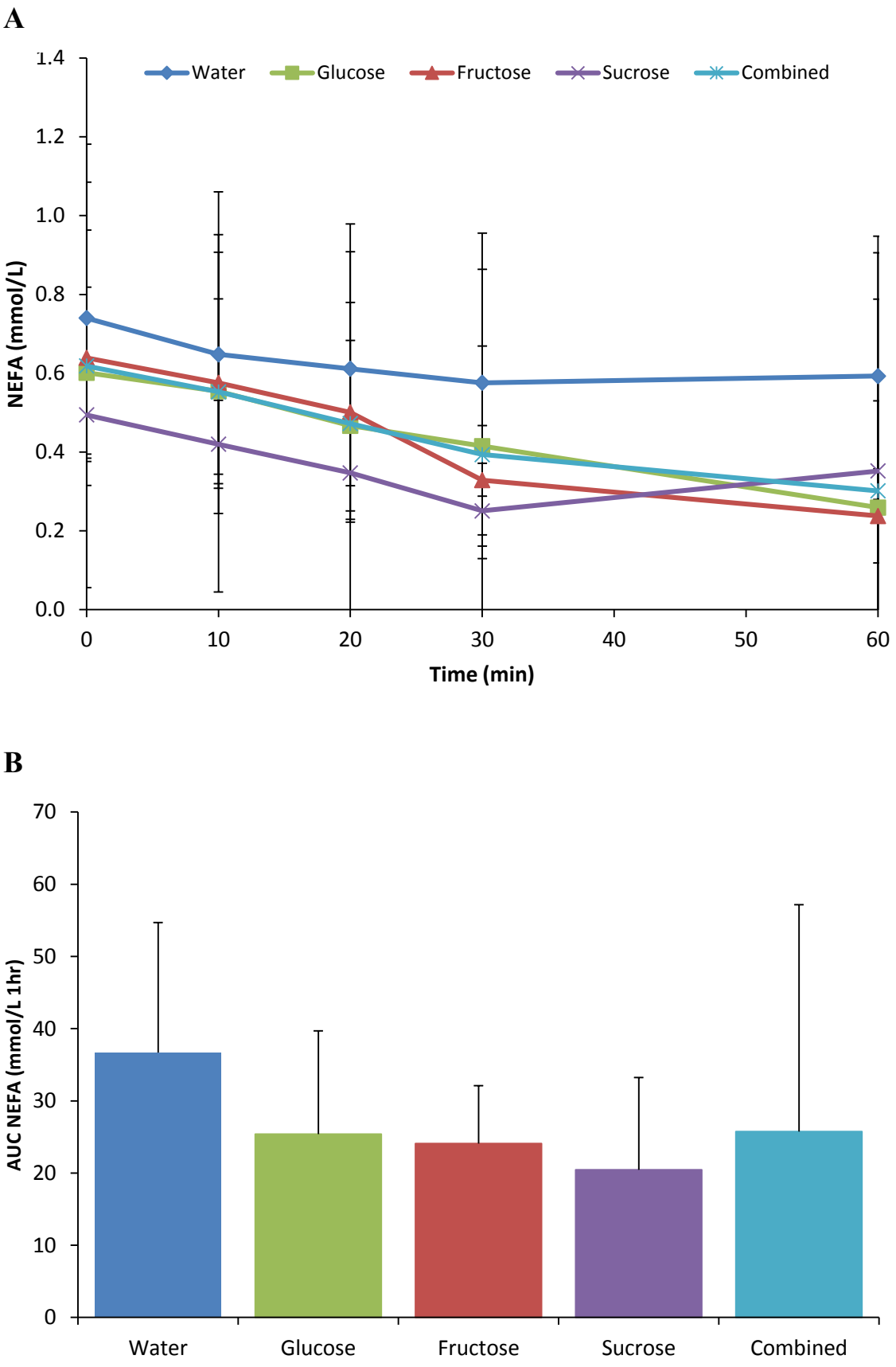

Figure 15. Serum non-esterified fatty acid (NEFA) concentration. (A) Response over 60 min and (B) Area under curve post ingestion of 595 mL water, 6% fructose, 6% glucose, 6% sucrose and 6% combined glucose and fructose solutions. Values are mean ± SD (*n* 7).

3.4. DISCUSSION

No significant differences in gastric emptying rate were found except for a higher T_{lag} for GLU compared with SUC, suggesting that gastric emptying of an isoenergetic glucose is slower than a sucrose solution. However, there was considerable inter-individual and between-trial variation in $T_{½}$, which may be partially explained by differences in gut hormone responses. Given that no subsequent effects were found for subjective appetite scores, this perhaps demonstrates a greater role of the investigated gut hormones in the regulation of gastric emptying than the direct regulation of appetite when ingesting fluids of this nature. The lack of statistical significance in gastric emptying data may have resulted from interactions of a large number of comparisons and a relatively small sample size. Statistical significance may be reached upon a decrease and/or increase of either of these factors, respectively. Having a much larger sample size may also reduce the impact or influence of the very large inter-individual variation seen particularly with the rate of emptying of glucose alone but also the other glucose containing solutions. This inter-individual variation may be a result of gastrointestinal adaptation to differing amounts of glucose in the diet or genetics, which will be investigated further.

A third possibility involves a very small doubt in the reliability of the breath test data. During data and sample collection from laboratory trials, an unforeseen complication with the breath sample analyser meant that breath samples were stored for a much longer period of time than anticipated before analysis. This led to very low concentrations of total CO_2 in the samples at the time of analysis. However, a small investigation (Appendix 2) on the effect of sample storage time length and decreased CO_2 concentration on the reproducibility and reliability of analysis revealed that the results of the current study can be accepted with a high degree of accuracy and certainty.

As expected, water, a non-nutrient liquid with the lowest osmolality, was observed to empty more quickly than any of the carbohydrate solutions. With the four carbohydrate solutions, fructose emptied arithmetically the fastest, followed by combined, then sucrose and lastly glucose. These results support the fructose, glucose and sucrose results reported by Elias *et al*. (1968) and Horowitz *et al*. (1996), and the combined glucose and fructose, and glucose results of Jeukendrup & Moseley (2010). The variation between the four carbohydrate solutions, however, cannot be attributed to the common variables such as volume, osmolality, and energy content. All ingested solutions were of identical volume and energy content, whilst FRU, GLU+FRU and GLU also all had the same osmolality. Sucrose, due to its disaccharide form has a lower osmolality, yet it did not have the second fastest $T_{½}$ emptying rate after water. This is consistent with the fact that the effect of

osmolality is less marked at lower concentrations of carbohydrate (Vist & Maughan, 1995). Sucrose did however have the lowest T_{lag} of all solutions, indicating an initial quick emptying rate before a slowing of emptying following intestinal sensing of its constituent monosaccharides after hydrolysis by sucrase located bound to the brush border of the intestinal mucosa (Miller & Crane, 1961).

This slowing of emptying is supported by GLP-1 hormone response to sucrose. The highest peak concentration observed was in response to SUC ingestion at approximately 20 min. Breath DOB data for sucrose shows a gradual decline in emptying from approximately 20 min. This provides evidence for the 'ileal brake' effect of GLP-1 also shown by Kong *et al*. (1999) and in conjunction with the different rates of emptying observed with different carbohydrate solutions indicates a sensing mechanism more interactive than purely osmoreceptors and energy nutrient sensors in the gastrointestinal tract.

The ingestion of fructose alone and the presence of fructose with glucose accelerated the emptying rate of a solution. As all four carbohydrates produced similar ghrelin suppression responses, it is unlikely that this increased rate of emptying with fructose was due to this orexigenic hormone. There were significant and marked differences between carbohydrates with GIP and GLP-1 response, respectively however. Fructose induced a GIP response significantly lower than the other carbohydrate solutions, which is comparable to the effects of water. On the other hand, whilst total GLP-1 response was not significantly lower for FRU compared to the other carbohydrates as might have been expected based on previous literature (Kong *et al*., 1999), noticeable differences in the pattern of response can be observed and may account for the accelerated emptying of FRU. Peak GLP-1 response for FRU was lower and occurred later in the trial at approximately 30 min compared to the other trials. Thus, a delay in the rise of GLP-1 and a reduced response would result in a less pronounced and delayed 'ileal brake' effect.

The ingestion of GLU and the ingestion of FRU resulted in respective increases in blood glucose concentration and blood fructose concentration in a dose-dependent manner. The blood glucose response to different carbohydrates was mirrored by both insulin and GIP responses. Interestingly, the pattern of response for GLP-1 did not also follow. In contrast to a widely held thought that GLP-1 plays a more potent role in glucose stimulated insulin release, the results of this study suggest a predominant role of the incretin GIP instead.

Serum lactate production significantly increased as a result of acute fructose ingestion and was significantly greater than the increase seen with glucose ingestion. This

increase occurred even with a relatively small amount of fructose of 18g within the SUC and GLU+FRU trials. Interestingly, SUC and GLU+FRU had a slightly greater and similar AUC, respectively, than FRU alone despite containing half of the amount of fructose in comparison. This is likely due to the different fates of fructose upon its metabolism. The presence of glucose in the ingested solutions may have led to preferential oxidation of glucose within the Krebs cycle as well as conversion to glycogen and thus limiting this pathway for fructose oxidation and resulting in greater lactate production. It is unlikely that this was due to reduced insulin action which is reported to result in less pyruvate entering the mitochondria for oxidation and thus cause a corresponding increase of anaerobic metabolism to lactate (Mueller, Stanhope, Gregoire, Evans & Havel, 2000) as insulin secretion following both sucrose and combined trials were pronounced in comparison to fructose. Another possible theory is related to the fact that fructose absorption is augmented when ingested with glucose (Truswell, Seach & Thorburn, 1988). However, it is unlikely that the observed results were due to greater or more efficient absorption of fructose when co-ingested with glucose, as serum fructose concentration was much greater following single 36 g fructose ingestion compared to the dual fructose-glucose solutions.

Triglyceride concentration was unchanged and was not significantly different between trials suggesting the acute ingestion of simple sugars in typical amounts does not result in immediate increased rates of de novo lipogenesis. It may be however that the 60 min sampling period was not long enough to detect any changes as triglyceride concentrations have been shown to be significantly elevated 2-3 hours after fructose ingestion (El-Sayed, MacLaren & Rattu, 1997; Bohannon, Karam & Forsham, 1980). In addition, whilst statistically significant decreases in NEFA concentration for W, GLU and GLU+FRU trials were observed, no differences in NEFA nor D-3-hydroxybutyrate concentration suppression was seen between carbohydrate trials indicating the ingestion of the different sugars resulted in similar reductions in lipolysis and NEFA metabolism. This is consistent with the studies by Ngo Sock *et al*. (2010), Teff *et al*. (2009) and Teff *et al*. (2004). For the trials involving glucose ingestion this is consistent with the elevation and action of insulin. However, this is unlikely to be the mechanism for reduced NEFA concentrations following fructose ingestion as insulin secretion is relatively unchanged. The mechanism in relation to this therefore seems unclear. Lastly, ingestion of single boluses of simple sugars in typical amounts had no effect on hepatic function, which suggests the deleterious effects of sugar consumption on hepatic function seen by Chiu *et al*. (2014) may not be due to additive effects of repeated single ingestions.

In conclusion, the results of this study did not show a statistically significant effect of carbohydrate type on gastric emptying rate apart from a significantly higher T_{lag} for GLU compared to SUC. Variation in emptying rates between the carbohydrates can be seen, however. No effect of carbohydrate type was also seen for hunger and appetite perceptions. The different carbohydrate types induced marked and significantly different hormone responses, however. Differences in the pattern of response of GLP-1 may be responsible for the observed variation in gastric emptying rate whilst differences in the pattern of response of GIP appears to have a greater incretin role. Ingestion of a single acute simple sugar solution containing typical amounts of sugar does not result in significantly increased triglyceride synthesis nor decreased hepatic function over the postprandial period investigated. Furthermore, no differences between sugars in these smaller quantities utilised were seen for lipolysis and NEFA metabolism suppression but fructose ingestion results in significantly increased lactate production which is augmented with glucose co-ingestion.

4. THE EFFECT OF SHORT-TERM DIETARY SUPPLEMENTATION WITH FRUCTOSE ON GASTRIC EMPTYING OF GLUCOSE AND FRUCTOSE[2]

[2] The data from this study contained within this chapter has been accepted for publication in "Yau, A.M.W., McLaughlin, J., Maughan, R.J., Gilmore, W., & Evans, G.H. (In Press). Short-term dietary supplementation with fructose accelerates gastric emptying of a fructose but not a glucose solution. *Nutrition*, http://dx.doi.org/10.1016/j.nut.2014.03.023". A copy of the accepted manuscript can be found at the back of this thesis. Some preliminary data from a number of participants was also presented as a poster communication and the abstract published in "Yau, A., McLaughlin, J., Maughan, R.J., Gilmore, W. & Evans, G.H. (2012). The effect of short-term dietary supplementation of fructose on gastric emptying of glucose and fructose. *Proceedings of the Nutrition Society*, 71 (OCE2), E133."

4.1. INTRODUCTION

A small compilation of research indicates that gastric emptying in humans may be influenced by patterns of previous dietary nutrient intake. Furthermore, there is evidence to suggest that these adaptive changes are macronutrient-specific specific (Castiglione *et al.*, 2002; Cunningham, Horowitz & Read, 1991) and rapid, with adaptations occurring in as little as three days (Clegg, McKenna, McClean, Davison, Trinick, Duly *et al*., 2011; Cunningham *et al*., 1991). A high fat diet for 14 d has been shown to accelerate gastric emptying of a high fat test meal (Cunningham, Daly, Horowitz & Read, 1991) but not a high-carbohydrate meal (Castiglione *et al*., 2002). More recently, this adaptive response of the gastrointestinal system to the ingestion of a high-fat meal has been reported to occur following only 3 d of high fat diet (Clegg *et al*., 2011). Similarly, short-term dietary supplementation with 400 g glucose per day for 3 d in healthy subjects has been shown to accelerate gastric emptying of hyperosmotic glucose solutions, but not a protein solution (Cunningham *et al*., 1991). The specificity of these effects of a high-glucose diet has not been extended to different monosaccharides, however. The emptying of a hyperosmotic fructose solution was equally accelerated following short-term supplementation with glucose solutions (Horowitz *et al*., 1996). Whether these effects are replicated in response to short-term dietary supplementation with fructose is unknown. The aim of this study was to investigate the effect of 3 d dietary fructose supplementation on the rate of gastric emptying of glucose and the rate of gastric emptying of fructose solutions as well as the accompanying subjective feelings of appetite.

4.2. METHODS

4.2.1. Participants

Ten healthy men completed this study (mean ± SD, age 27 ± 6 years, height 179.9 ± 9.2 cm, BM 81 ± 11 kg, BMI 25 ± 3 $kg.m^{-2}$, and estimated body fat percentage 21 ± 8%). Written informed consent was obtained from all participants.

4.2.2. Experimental trials

Participants reported to the laboratory on four occasions to complete four experimental trials; fructose with supplementation (FS), fructose with water control (FC), glucose with supplementation (GS) and glucose with water control (GC). Experimental trials were conducted in a single-blind, randomised crossover fashion and each separated by a minimum period of 7 d. In addition to the pre-trial conditions outlined in general methods, each experimental trial was preceded by a 3 d dietary and activity maintenance period

where participants were asked to record their diet and activity in their first trial and then replicate them in the remaining three trials. The purpose of this was to ensure standardisation and consistency of macronutrient intake and metabolic status leading up to each trial within participants. In addition to their normal dietary intake, participants were asked to consume either four 500 mL bottles of water or four 500 mL solutions each containing 30 g fructose per day over the 3 d. Participants were instructed to consume these drinks evenly throughout the day in between meals.

Upon arrival at the laboratory, participants were asked to completely empty their bladder into a container from which a 5 mL urine sample was retained for later analysis of osmolality. Body mass was subsequently recorded. Participants then ingested 595 mL of a fructose solution (36 g dissolved in water to a volume of 600 mL) or an equicaloric glucose monohydrate solution (39.6 g dissolved in water to a volume of 600 mL). Both glucose and fructose were purchased from MyProtein (www.myprotein.com) and water purchased from a local supermarket (Evian, Danone Ltd, France). Participants were given a maximum of 2 min to consume the test solution and instructed to consume it as quickly as they were able to. Test drink solutions were freshly prepared on the morning of the test and were given at room temperature. A 5 mL sample of the drink was retained for later analysis of osmolality. Participants remained seated throughout the drink ingestion and 60 min sampling procedure. Gastric emptying rate and appetite was assessed for the 60 min duration of the study as described in general methods. Following all sample collections at 60 min, participants were asked again to completely empty their bladder into a container and a 5 mL urine sample was again retained for later analysis.

4.2.3. Statistical analysis

Differences in pre-ingestion BM, pre-ingestion urine osmolality and drink osmolality were examined using one-way repeated ANOVA. Two-way repeated ANOVA were used to examine differences in gastric emptying DOB values, and subjective appetite VAS scores. Sphericity for repeated measures was assessed, and where appropriate, Greenhouse-Geisser corrections were applied for epsilon < 0.75, and the Huynh-Feldt correction adopted for less severe asphericity. Significant *F*-tests were followed by repeated one-way ANOVA and bonferroni adjusted pairwise comparisons as appropriate. Gastric emptying $T_{½}$ and T_{lag} data were examined with paired Student's *t*-Tests to test the hypothesis of interest (i.e. effect of supplementation on gastric emptying rate of fructose and of glucose). Paired Student's *t*-tests were also used to directly compare gastric emptying $T_{½}$ and T_{lag} of fructose and glucose control and supplementation trials. All data were analysed using SPSS

Statistics for Windows version 19 (IBM, New York, US). Statistical significance was accepted at the 5% level and results presented as means and standard deviations.

4.3. RESULTS

4.3.1. Body mass, hydration status and drink osmolality

Body mass remained stable over the duration of the study (Table 3). Furthermore, the constancy of pre-ingestion urine osmolality indicated that hydration status prior to each experimental trial was also consistent (Table 3). Drink osmolalities were 368 ± 3, 368 ± 3, 370 ± 4 and 369 ± 3 mOsmol.kg^{-1} (P = 0.490) for FC, FS, GC and GS, respectively.

Table 3. Pre-trial body mass and hydration marker (n 10).

	Fructose		**Glucose**		***P*-value**
	Control	Supplement	Control	Supplement	
Body mass (kg)	80.91 ± 11.48	81.23 ± 11.53	81.80 ± 11.70	81.03 ± 11.38	0.589
Urine osmolality (mOsmol/kg)	423 ± 259	489 ± 265	425 ± 230	452 ± 270	0.613

4.3.2. Gastric emptying

Gastric emptying $T_{½}$ for fructose was accelerated after the period of dietary supplementation with fructose than when the control water was consumed (FC, 58 ± 14 min vs. FS, 48 ± 6 min; P = 0.037). In contrast, gastric emptying $T_{½}$ for glucose did not change with fructose supplementation (GC, 78 ± 27 min vs. GS, 85 ± 31 min; P = 0.273). The same pattern was also observed for T_{lag}. Dietary fructose supplementation accelerated fructose T_{lag} (FC, 38 ± 9 min vs. FS, 33 ± 6 min; P = 0.042) whilst glucose T_{lag} remained unchanged (GC, 44 ± 14 min vs. GS, 45 ± 14 min; P = 0.757). Breath DOB values for fructose (Figure 16) revealed no main effect of trial (P = 0.441), a significant main effect of time (P < 0.001) and an interaction effect tending to significance (P = 0.088). Breath DOB for glucose (Figure 17) showed no main effect of trial (P = 0.868), a significant main effect of time (P < 0.001) and no interaction effect (P = 0.680). Direct comparison between FC and GC emptying revealed fructose $T_{½}$ was significantly shorter than glucose (P = 0.039). No difference in T_{lag} was seen, however (P = 0.242). Direct comparison between FS and GS revealed fructose $T_{½}$ (P = 0.007) and T_{lag} (P = 0.033) were significantly shorter than that of glucose.

4.3.3. Appetite ratings

Hunger ratings for fructose trials remained relatively constant from baseline and over the 60 min duration after drink ingestion. No main effect of supplementation ($P = 0.820$), time ($P = 0.160$) or interaction ($P = 0.364$) was present. Ingestion of a glucose solution, on the other hand, resulted in a slight suppression of hunger within 10 min before a steady rise back to baseline values within 60 min. No statistically significant main effect of supplementation ($P = 0.861$), time ($P = 0.07$) or interaction effect ($P = 0.562$) were identified (Figure 18).

Ingestion of a fructose solution did not affect ratings of fullness over the 60 min (FC, $P = 0.130$; FS, $P = 0.137$). Prior fructose supplementation also did not affect ratings of fullness when compared with its control as no main effect of supplementation ($P = 0.135$) and no interaction effect ($P = 0.706$) were found. Ratings of fullness following glucose ingestion were also not different between control and supplementation trials. No main effect of supplementation ($P = 0.575$) or interaction ($P = 0.285$) was present, though a biphasic increase then decrease in fullness following glucose ingestion with prior supplementation was observed compared to the single increase then decrease seen with no supplementation (Figure 19). A significant main effect of time was indicated ($P = 0.004$), though post-hoc analysis did not identify the location.

Prospective food consumption decreased slightly within 10 min of ingestion of a fructose solution. For the control trial, this steadily increased back to pre-ingestion value within 60 min. For the supplementation trial, an increase above pre-ingestion values was seen at 50 and 60 min. A main effect of time ($P = 0.011$), but no significant effects of trial ($P = 0.344$) or interaction ($P = 0.205$), was found. Significant differences between ratings over time were not located with post-hoc analysis. A similar decrease followed by a gradual increase back to baseline scores was also seen for the ingestion of glucose for both control and supplementation conditions. Again, no effect of trial ($P = 0.898$) nor interaction ($P = 0.142$) was shown, but there was an effect of time ($P = 0.048$).

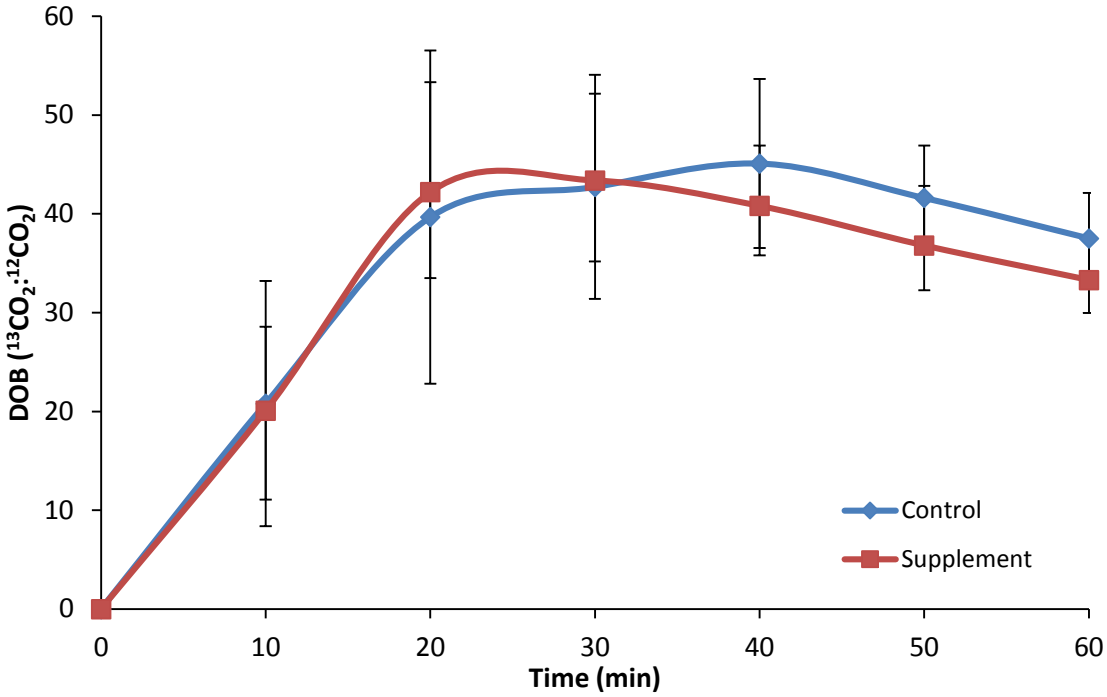

Figure 16. Gastric emptying breath delta over baseline (DOB) for 60 min following 595 mL 6% fructose solution ingestion. Treatments were control without fructose supplementation and with 3 d supplementation of 120 g fructose per day. Values are mean ± SD (*n* 10).

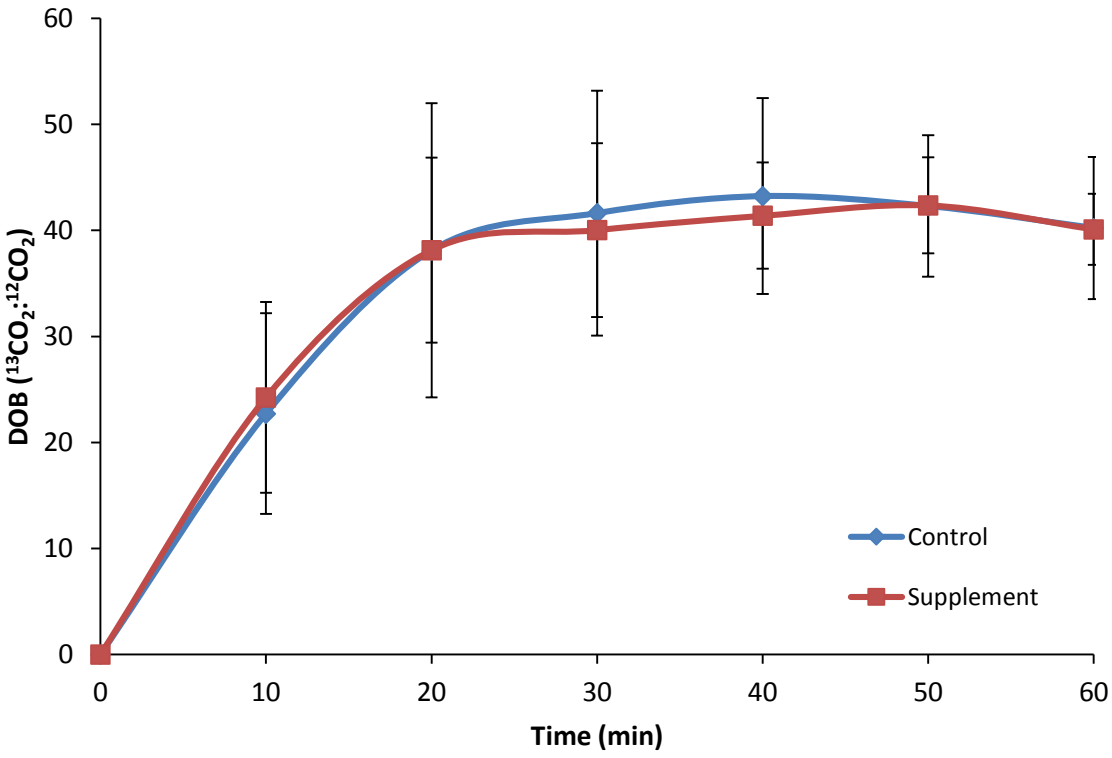

Figure 17. Gastric emptying breath delta over baseline (DOB) for 60 min following 595 mL 6% glucose solution ingestion. Treatments were control without fructose supplementation and with 3 d supplementation of 120 g fructose per day. Values are mean ± SD (*n* 10).

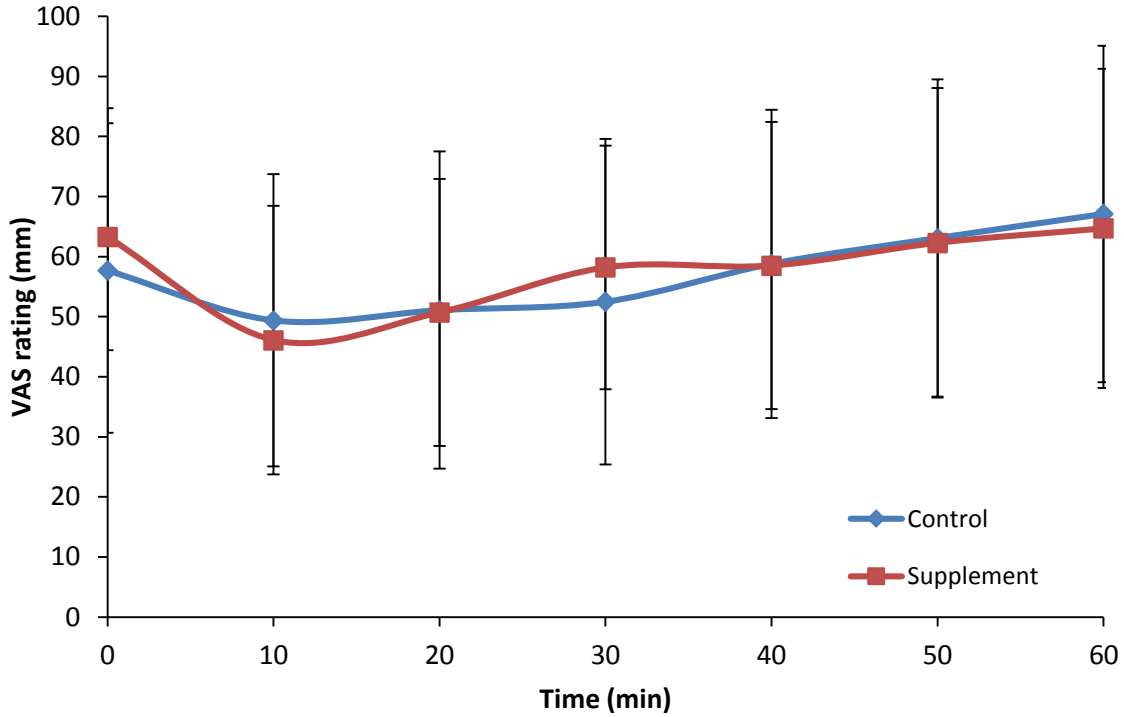

Figure 18. Subjective feeling of hunger assessed by 100-mm visual analogue scale (VAS) for 60 min following ingestion of 595 mL of a 6% fructose solution. Treatments were control without fructose supplementation and with 3 d supplementation of 120 g of fructose per day. Values are means ± SD (*n* 10).

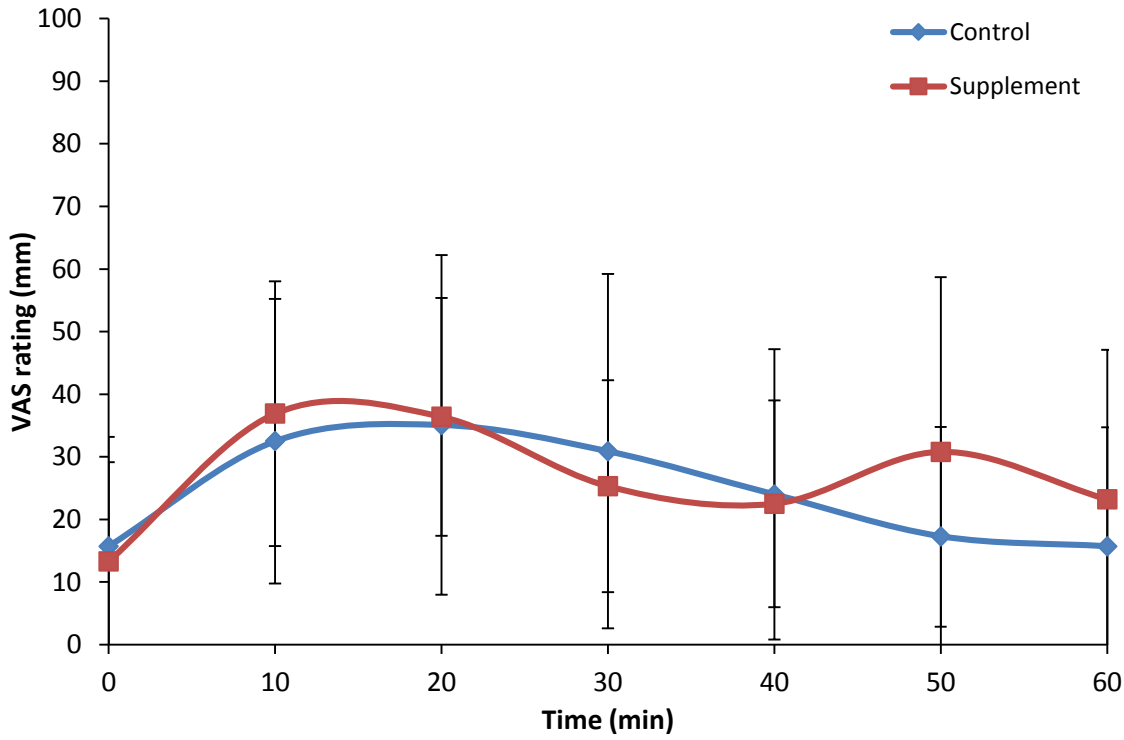

Figure 19. Subjective feeling of fullness assessed by 100-mm visual analogue scale (VAS) for 60 min following ingestion of 595 mL of a 6% glucose solution. Treatments were control without fructose supplementation and with 3 d supplementation of 120 g of fructose per day. Values are means ± SD (*n* 10).

4.4. DISCUSSION

The results of this study show that a 3-d period of dietary supplementation with 120 g fructose consumed throughout the day results in an acceleration of gastric emptying of a fructose solution but not of a glucose solution. This study thus shows a monosaccharide-specific adaptation to increased fructose in the diet in contrast to the glucose supplementation results of Horowitz *et al.* (1996). Furthermore, the results of this present study demonstrate an adaptation of gastric emptying rate to a much smaller amount of additional carbohydrate consumption than that utilised in previous studies, and highlight the pertinent potential negative effects of an increase in dietary fructose consumption. An amount of 30 g of fructose is on average less than the amount that would be found in a typical 500 mL serving of commercially-available soft drinks which contain 11.0-12.5% high fructose corn syrup (55% fructose) in some countries such as the US. The fructose content in the majority of these soft drinks thus range from a little over 30 g to 34 g. Although the dose of fructose ingested in this study (120g/day) is four times the amount of this typical single serving, data shows that it is not an unrealistic amount. Estimated daily mean, 90th and 95th percentile fructose intakes from NHANES data are reported respectively as 63 g, 103 g and 118 g for males aged 23-50 y and 75 g, 117 g and 134 g for males aged 19-22 y (Marriott, Cole & Lee, 2009).

The increased rate of gastric emptying following fructose supplementation is highly indicative of a short-term reduction in gastric emptying inhibition resulting from small intestinal feedback. This may have been due to several possible adaptations. One possible mechanism is a decreased sensitivity to fructose by specific receptors in the small intestine. However, the existence of fructose-selective receptors has not been reported and is perhaps rather unlikely. Another possible mechanism is an enhanced absorption capacity of the small intestine for fructose, resulting in decreased intestinal exposure time and length, may have occurred. The length of intestine exposed to nutrients has been shown to be an important determinant of the extent of feedback inhibition of gastric emptying (Lin, Doty, Reedy & Meyer, 1990; 1989). Alternatively, and/or in combination with this, the adaptation of enhanced absorption leading to augmented transporter activation may be responsible. This latter explanation seems more plausible in the light of the current study's monosaccharide-specific results due to the different transport pathways of fructose and glucose. Glucose is actively transported across the brush border membrane of the intestine by sodium-dependent glucose transporter 1 (SGLT1) and across the basolateral membrane by the GLUT2 hexose transporter (Levin, 1994). Fructose, however, is absorbed through facilitated transport by a sodium-independent transport system, believed to primarily be

the GLUT5 transporter, and across the basolateral membrane also by GLUT2 (Jones, Butler & Brooks, 2011; Levin, 1994). The different yet inter-related monosaccharide effects of the present study and that of Horowitz *et al*. (1996) are consistent with an upregulation of GLUT5 activity in response to dietary fructose supplementation and an upregulation of both glucose and fructose transport pathways (possibly involving GLUT2) following increased dietary glucose exposure. In any case, as nutrient transporters appear to have a role in nutrient sensing and gut hormone secretion (Raybould, 2008; Gribble, Williams, Simpson & Reimann, 2003), this may have led to changes in either the secretion of or sensitivity to gut hormones such as GLP-1 or ghrelin, both of which are known to affect the rate of gastric emptying. Previous work investigating the effect of acute ingestion of fructose on gastrointestinal response is limited and with specific regards to GLP-1 and ghrelin is conflicting. Some have reported fructose to stimulate GLP-1 (Kong *et al*., 1999), insulin (Bowen *et al*., 2007; Teff *et al*., 2004; Kong *et al*., 1999), and leptin (Teff *et al*., 2004) secretion, and suppress ghrelin (Teff *et al*., 2004) to a lesser degree than comparable amounts of glucose. Others, including the data presented in chapter 3 of this thesis, have seen similar GLP-1 and ghrelin responses (Bowen *et al*., 2007). No data is currently available on repeated ingestion or the effects of short-term increases or habitually high intakes of fructose in humans. Further work investigating whether any changes in gut hormone responses occur with fructose supplementation is required to elucidate the mechanism of gastrointestinal adaptation observed in this present study.

The ingestion of a single bolus of fructose results in markedly lower plasma glucose and insulin responses compared to the response following an isoenergetic amount of glucose or sucrose (Kong *et al*., 1999; Horowitz *et al*., 1996; Bohannon *et al*., 1980; Crapo, Kolterman & Olefsky, 1980). Whilst this may be beneficial in the short-term postprandial maintenance and control of blood glucose levels in diabetics, this also has negative appetite regulation and metabolic consequences irrespective of insulin status. Decreased insulin secretion and production results in decreased circulating levels of leptin, the long term regulator of food intake, and reduced suppression of the orexigenic hormone ghrelin (Teff *et al*., 2004). Glucagon suppression is also significantly lower following fructose ingestion leading to greater glycogenolysis and lipolysis and increased plasma triglyceride concentrations (Bohannon *et al*., 1980). Furthermore, the complete metabolism of fructose in hepatocytes results in an unregulated source of substrates for augmented *de novo* lipogenesis (Stanhope *et al*., 2009; Elliott *et al*., 2002) and also increased uric acid concentration (Johnson *et al*., 2007). Accelerated gastric emptying of fructose would therefore lead to more rapid rises in plasma fructose and may result in both larger and

earlier peaks of plasma triglycerides and uric acid, both of which are strong independent contributors to the development of diabetes, cardiovascular disease, and obesity (Johnson *et al*., 2007; Elliott *et al*., 2002).

Although no significant changes to appetite ratings were observed in this present study, this is likely due to the fact that ingestion of liquids generally provides a smaller satiation effect than does ingestion of isoenergetic solids (Martens & Westerterp-Plantenga, 2012; Pan & Hu, 2011). The effect of increased fructose ingestion on gastrointestinal adaptation and appetite should also be investigated in solid foods.

In conclusion, the results of present study reveal that three consecutive days of dietary supplementation with 120 g fructose per day accelerates gastric emptying of a fructose solution but not of a glucose solution. These monosaccharide-specific results are in contrast to previous research with glucose supplementation, and indicate a potential deleterious adaptation by which repeated dietary fructose loads may contribute to the development of obesity and the metabolic syndrome. The mechanisms and implications of this observed gastrointestinal adaptation to increased dietary fructose should be further investigated.

5. THE EFFECT OF SHORT-TERM DIETARY SUPPLEMENTATION WITH FRUCTOSE ON GASTRIC EMPTYING OF GLUCOSE AND FRUCTOSE AND ASSOCIATED GUT HORMONE AND TRIGLYCERIDE RESPONSES[3]

[3] Some of the data from this study contained within this chapter was presented as a poster communication and the abstract published in "Yau, A., McLaughlin, J., Maughan, R.J., Gilmore, W. & Evans, G.H. (2014). The effect of short-term dietary supplementation with fructose on gastric emptying of glucose and fructose and associated gut hormone responses. *International Journal of Sport Nutrition and Exercise Metabolism*, 24, S5."

5.1. INTRODUCTION

It was previously shown in Chapter 4 that 3 d of dietary supplementation with fructose results in a monosaccharide specific acceleration of gastric emptying rate. As discussed, one potential mechanism for this adaptation is a change in gut hormone response. Furthermore, alterations in the secretion of gut hormones may have important implications in the regulation of energy intake as well as gastrointestinal function (Little *et al*., 2007). A small number of previous studies that have investigated the effects of previous dietary intake on gut hormone responses have shown changes in the secretion of gut hormones such as CCK, GLP-1, PYY and ghrelin. The majority of the work to date has been conducted on the effects of a high fat diet, however, and few have simultaneously measured gastric emptying rate.

Following the observations by Cunningham *et al*. (1991) where emptying rate of a fatty meal was accelerated as a result of a high fat diet for two weeks, investigations on CCK responses were conducted. Studies by French, Murray, Rumsey, Fadzlin & Read (1995) and Spannagel, Nakano, Tawil, Chey, Liddle & Green (1996) in humans and rats, respectively, were the earliest of investigations to report an increase in postprandial CCK concentration following a high-fat diet. Gastric emptying was not assessed in this study, however, and though a discrepancy exists in the effects of a high fat diet on gastric emptying rate, other data suggests that moderations of gut hormone response following a high fat diet is associated and may be consistent with the observed adaptations in gastric emptying rate. Notably, eight weeks of a high fat diet in rats resulted in slower gastric emptying which was associated with lower plasma ghrelin concentration and higher CCK and leptin responses (Li, Ma & Wang, 2011). Fasting levels of CCK have also been shown to be altered in humans following a high fat diet. Increased fasting levels of CCK but not PYY nor ghrelin has been reported to result after 21 d of a high fat diet compared with an isoenergetic low fat diet (Little, Feltrin, Horowitz, Meyer, Wishart, Chapman *et al*., 2008). The effect of a high fat diet has also been shown by others to suppress ghrelin response to a greater extent in rats (Beck, Musse & Stricker-Krongrad, 2002; Lee, Wang, Englander, Kojima & Greeley, 2002) and humans (Robertson, Henderson, Vist & Rumsey, 2004), reduce fasting and postprandial PYY concentrations in mice (le Roux *et al*. 2006), and increase fasting levels and postprandial GLP-1 secretion in dogs (van Citters, Kabir, Kim, Mittelman, Dea, Brubaker *et al*., 2002) but not humans (Boyd, O'Donovan, Doran, Wishart, Chapman, Horowitz *et al*., 2003). Acceleration of gastric emptying of a protein containing meal following a high protein diet for two weeks has also been shown to result

in significantly lower postprandial CCK in rats compared to those fed a low or medium protein diet (Shi , Leray, Scarpignato, Bentouimou, desVarannes, Cherbut *et al*., 1997).

With regards to increased dietary intake of carbohydrates and moderation of gut hormone responses, very few studies are currently present in the literature. Of those available, high glucose intake for 4-7 d resulted in accelerated gastric emptying of glucose and fructose but differential gut hormones responses (Horowitz *et al*., 1996). Greater GIP responses were observed following the glucose supplemented diet for both carbohydrates. However, insulin response was greater in the glucose load but unchanged in the fructose load in the glucose supplemented trials (Horowitz *et al*., 1996). Furthermore, glycaemic response was lower for a glucose load but not a fructose load following glucose supplementation (Horowitz *et al*., 1996). The only two studies to our knowledge that have investigated the effects of increased fructose consumption on gut hormones showed that two weeks of a high fructose diet in rats increased fasting ghrelin levels by 40% (Lindqvist *et al*., 2008), and four weeks of a high fructose diet in healthy men increased fasting leptin concentrations within the first week (Le *et al*., 2006). The effect of increased fructose consumption on moderations of postprandial gut hormone responses in relation to adaptations of gastric emptying rate is therefore unknown.

In addition, chapter 3 showed little differential effects on hepatic metabolism and function besides lactate production following the acute ingestion of different simple sugars in amounts reflective of a typical serving. As previously mentioned, increased fructose ingestion for 1 d to 6 weeks has been shown to result in increased fasting and postprandial plasma triglyceride concentrations when compared to glucose ingestion (Stanhope *et al*., 2011; Ngo Sock *et al*., 2010; Stanhope *et al*., 2009; Teff *et al*., 2009; Stanhope *et al*., 2008; Teff *et al*., 2004; Bantle *et al*., 2000). The effect of a relatively shorter period of increased fructose intake is unknown. Therefore, the aim of this study was to investigate the associated gut hormone responses and hepatic lipogenesis effects of a short-term increase in dietary fructose ingestion.

5.2. METHODS

5.2.1. Participants

Ten healthy men completed this study (mean ± SD, age 26 ± 7 y, height 179.0 ± 6.3 cm, BM 81 ± 11 kg, BMI 25 ± 3 $kg.m^{-2}$, and estimated body fat percentage 23 ± 8%).

5.2.2. Experimental trials

As with the study in chapter 4, participants reported to the laboratory on four occasions to complete four experimental trials; fructose with supplementation (FS), fructose with water control (FC), glucose with supplementation (GS) and glucose with water control (GC). Experimental trials were separated by a minimum period of 7 d. In addition to the pre-trial conditions outlined in general methods, each experimental trial was preceded by a 3 d dietary and activity maintenance period where participants were asked to record their diet and activity in their first trial and then replicate them in the remaining three trials. In addition to their normal dietary intake, participants were asked to consume either four 500 mL bottles of water or four 500 mL solutions each containing 30 g fructose per day over the 3 d. Participants were instructed to consume these drinks evenly throughout the day in between meals.

Upon arrival at the laboratory, participants were asked to completely empty their bladder into a container from which a 5 mL urine sample was retained for later analysis of osmolality. Body mass was subsequently recorded. Following this either a 21 gauge or 22 gauge intravenous cannula (Venflon; Becton Dickinson, Plymouth, UK) was inserted into an antecubital vein and a catheter extension (Vygon, Ecouen, France) attached. A baseline blood sample was then obtained using the procedure outlined in general methods. Participants then ingested 595 mL of a fructose solution (36 g dissolved in water to a volume of 600 mL) or an equicaloric glucose monohydrate solution (39.6 g dissolved in water to a volume of 600 mL). Both glucose and fructose were purchased from MyProtein (www.myprotein.com) and water was purchased from a local supermarket (Evian, Danone Ltd, France). Participants were given a maximum of 2 min to consume the test solution and instructed to consume it as quickly as they were able to. Drink solutions were prepared fresh in the morning prior to the trial and given at room temperature and a 5 mL sample of the drink was retained for later analysis of osmolality. Participants remained seated throughout the drink ingestion and 60 min sampling procedure. Further blood samples were obtained at 10, 20, 30, 45 and 60 min post-drink ingestion and gastric emptying rate and appetite was assessed for the duration of study as described in general methods. Following all sample collections at 60 min, the cannula was removed and participants were asked again to completely empty their bladder into a container and a 5 mL urine sample was again retained for later analysis.

5.2.3. Biochemical analysis

In addition to the blood sample analysis described in general methods, the adipokine leptin was also determined using the human gut hormone multiplex assay (Milliplex MAP, Merck Millipore Ltd, UK). Gut hormone analysis was performed in duplicate for 88% of the samples. Intra-assay CVs for ghrelin, GIP, GLP-1, insulin and leptin were 5.0%, 4.8%, 17.6%, 4.4% and 3.7%, respectively. Inter-assay CVs for ghrelin, GIP, GLP-1, insulin and leptin were 16.6%, 13.2%, 14.0%, 7.5% and 7.1%, respectively. Glucose, lactate and triglyceride analysis was performed in duplicate for all samples. Intra-assay CVs were 1.3%, 1.4% and 1.5%, respectively. Intra-assay CV for fructose analysis with 26 samples analysed in duplicate was 5.1%.

5.2.4. Statistical analysis

Differences in pre-ingestion BM, pre-ingestion urine osmolality, drink osmolality, and gut hormone concentration AUC were examined using one-way repeated ANOVA. Significant *F*-tests were followed by Bonferroni adjusted pairwise comparisons. Two-way repeated ANOVA were used to examine differences in gastric emptying DOB values, urine osmolality, serum osmolality, blood glucose and fructose concentrations, gut hormone concentrations, and subjective appetite VAS scores. Significant *F*-tests were followed with the appropriate paired Student's *t*-Tests or one-way repeated ANOVA and Bonferroni adjusted pairwise comparisons. Sphericity for repeated measures was assessed, and where appropriate, Greenhouse-Geisser corrections were applied for epsilon < 0.75, and the Huynh-Feldt correction adopted for less severe asphericity. Gastric emptying $T_{½}$ and T_{lag} data were examined with paired Student's *t*-Tests to test the hypothesis of interest (i.e. effect of supplementation on gastric emptying rate of fructose and of glucose). Paired Student's *t*-tests were also used to directly compare gastric emptying $T_{½}$ and T_{lag} of fructose and glucose control and supplementation trials. All data were analysed using SPSS Statistics for Windows version 19 (IBM, New York, US). Statistical significance was accepted at the 5% level and results presented as means and SD.

5.3. RESULTS

5.3.1. Body mass, hydration status and drink osmolality

Body mass remained stable over the duration of the study (Table 4). Pre-ingestion urine osmolality were generally lower in each respective supplement trial but differences over the course of the study did not reach statistical significance (Table 4). Drink osmolalities were 368 ± 3, 367 ± 4, 371 ± 3 and 370 ± 4 mOsmol/kg (P = 0.010) for FC, FS, GC and

GS, respectively. No significantly different pairwise comparisons were located following the significant *F*-test.

Table 4. Pre-trial body mass and hydration marker (*n* 10).

	Fructose		**Glucose**		
	Control	Supplement	Control	Supplement	***P*-value**
Body mass (kg)	80.87 ± 11.15	81.13 ± 11.04	81.48 ± 11.46	80.95 ± 10.80	0.338
Urine osmolality (mOsmol/kg)	560 ± 262	397 ± 271	504 ± 266	356 ± 193	0.067

5.3.2. Gastric emptying

Gastric emptying $T_{½}$ for fructose was accelerated after the period of dietary supplementation with fructose than when the control water was consumed (FC, 59 ± 13 min vs. FS, 51 ± 10 min; $P = 0.004$). In contrast, gastric emptying $T_{½}$ for glucose did not significantly change with fructose supplementation (GC, 75 ± 18 min vs. GS, 68 ± 16 min; $P = 0.245$). The same pattern was also observed for T_{lag}. Dietary fructose supplementation accelerated fructose T_{lag} (FC, 37 ± 3 min vs. FS, 32 ± 7 min; $P = 0.026$) whilst glucose T_{lag} remained unchanged (GC, 38 ± 7 min vs. GS, 40 ± 7 min; $P = 0.679$). Breath DOB values for fructose (Figure 20) revealed no main effect of trial ($P = 0.912$), a significant main effect of time ($P < 0.001$) and no interaction effect ($P = 0.376$). Breath DOB for glucose (Figure 21) showed no main effect of trial ($P = 0.537$), a significant main effect of time ($P < 0.001$) and no interaction effect ($P = 0.282$). Direct comparison between FC and GC revealed a trend of a shorter gastric emptying $T_{½}$ for fructose ($P = 0.088$). T_{lag} was not different, however ($P = 0.696$). Direct comparison between FS and GS revealed fructose $T_{½}$ ($P = 0.016$) and T_{lag} ($P = 0.035$) were significantly shorter than glucose.

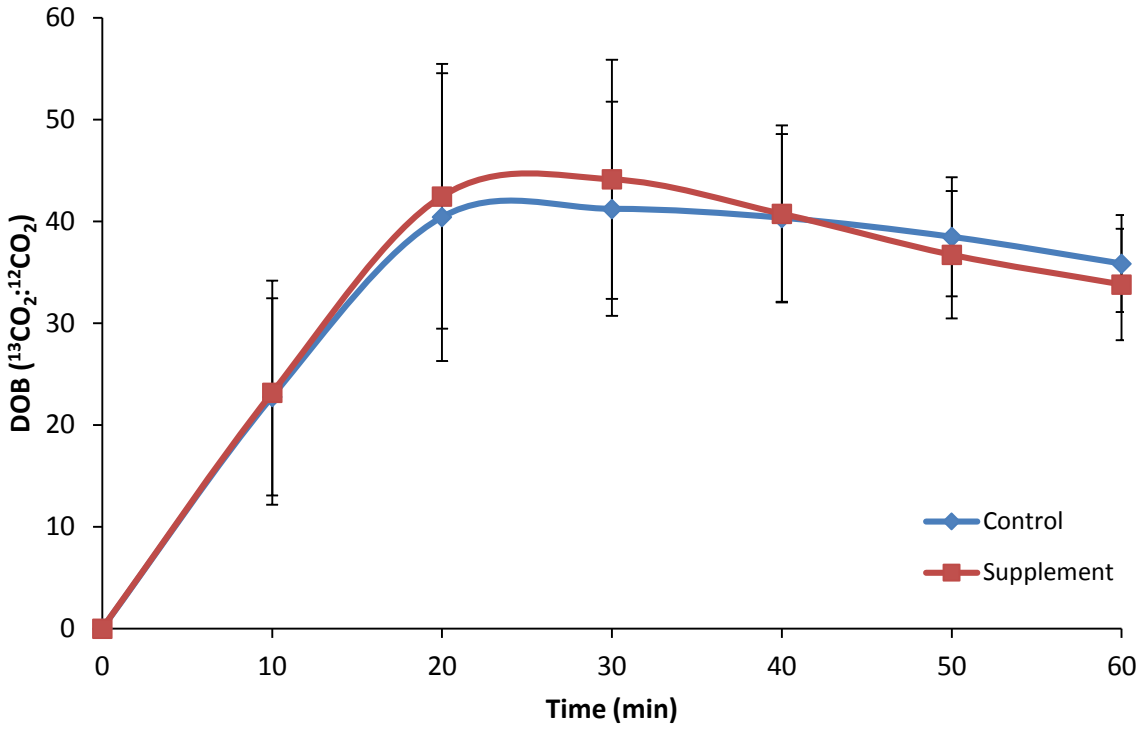

Figure 20. Gastric emptying breath delta over baseline (DOB) for 60 min following 595 mL 6% fructose solution ingestion. Treatments were control without fructose supplementation and with 3 d supplementation of 120 g fructose per day. Values are mean ± SD (*n* 10).

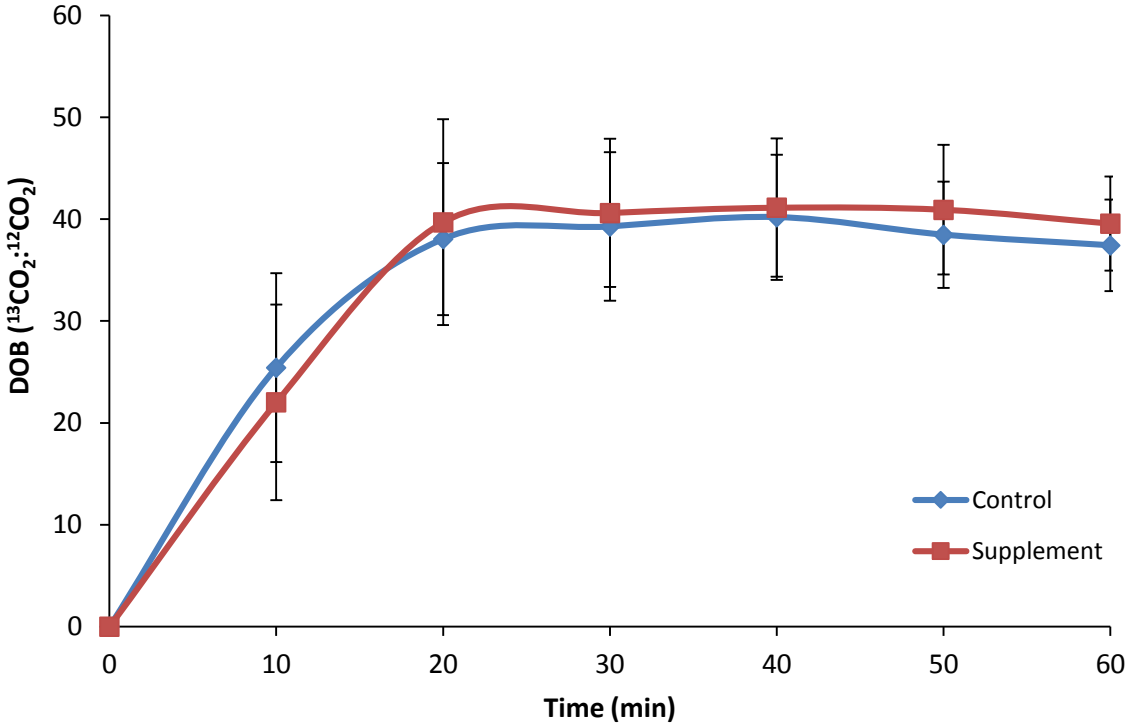

Figure 21. Gastric emptying breath delta over baseline (DOB) for 60 min following 595 mL 6% glucose solution ingestion. Treatments were control without fructose supplementation and with 3 d supplementation of 120 g fructose per day. Values are mean ± SD (*n* 10).

5.3.3. Gut hormones

5.3.3.1. Ghrelin

Baseline ghrelin concentrations were not different between all four trials (FC, 156.65 ± 77.25 pg/mL; FS, 174.64 ± 82.47 pg/mL; GC, 172.06 ± 68.19 pg/mL; GS 184.05 ± 71.00 pg/mL; $P = 0.131$) though there was a pattern for higher baseline levels following supplementation compared to each respective control trial. For fructose, this tended to significance ($P = 0.089$). Two way ANOVA revealed no main effect of trial ($P = 0.261$), a significant effect of time ($P < 0.001$) and an interaction effect ($P = 0.018$) for all four trials. Analysis of fructose ingestion (Figure 22a) revealed no main effect of supplementation ($P = 0.264$) but a significant effect of time ($P < 0.001$) and a trend of an interaction ($P = 0.065$). Post-hoc analysis revealed that ghrelin concentration significantly decreased between 10 min to 60 min in the control trial whilst the decrease in the supplement trial decreased from baseline values from 20 min post ingestion. A trend of significantly lower ghrelin was also indicated for supplementation compared to control at 45 min post ingestion ($P = 0.063$). Area under curve was not different ($P = 0.800$; Figure 22b). Analysis of glucose ingestion (Figure 23a) revealed a trend of a supplementation effect ($P = 0.080$), a significant effect of time ($P < 0.001$) and no interaction effect ($P = 0.276$). Post-hoc analysis showed ghrelin concentration significantly decreased from baseline levels at 20-60 min post ingestion in both trials and ghrelin concentration was significantly higher in the supplement trial compared to control at 10 min post ingestion ($P = 0.019$). Area under curve was not different ($P = 0.288$; Figure 23b).

5.3.3.2. GIP

Baseline GIP concentrations were not significantly different between all four trials (FC, 10.78 ± 12.44 pg/mL; FS, 8.26 ± 4.13 pg/mL; GC, 9.31 ± 8.18 pg/mL; GS, 12.47 ± 15.20 pg/mL; $P = 0.545$). Two way ANOVA for all four trials revealed a significant trial effect ($P = 0.001$), a significant main effect of time ($P = 0.005$) and a significant interaction effect ($P < 0.001$). Analysis for fructose ingestion (Figure 24a) showed no effect of supplementation ($P = 0.760$), time ($P = 0.121$) or interaction ($P = 0.368$). Area under curve was also not different ($P = 0.964$; Figure 24b). Analysis of glucose ingestion (Figure 25a) revealed a trend of a supplementation effect ($P = 0.076$), a significant effect of time ($P < 0.001$) but no interaction effect ($P = 0.707$). GIP concentration for GC significantly increased from baseline values rapidly at 10 min then decreased from 20 min but remained significantly higher than baseline at 60 min. On the other hand, GIP concentration for GS

significantly increased from baseline at 30 min and remained higher than baseline at 60 min but not significantly. Despite this, GIP concentration was significantly higher at 60 min compared to control (P = 0.049). There was also a trend for greater AUC for supplement compared to control (P = 0.072; Figure 25b). Responses for both glucose ingestion trials were significantly greater than both fructose ingestion trials at all post ingestion time-points ($P < 0.01$).

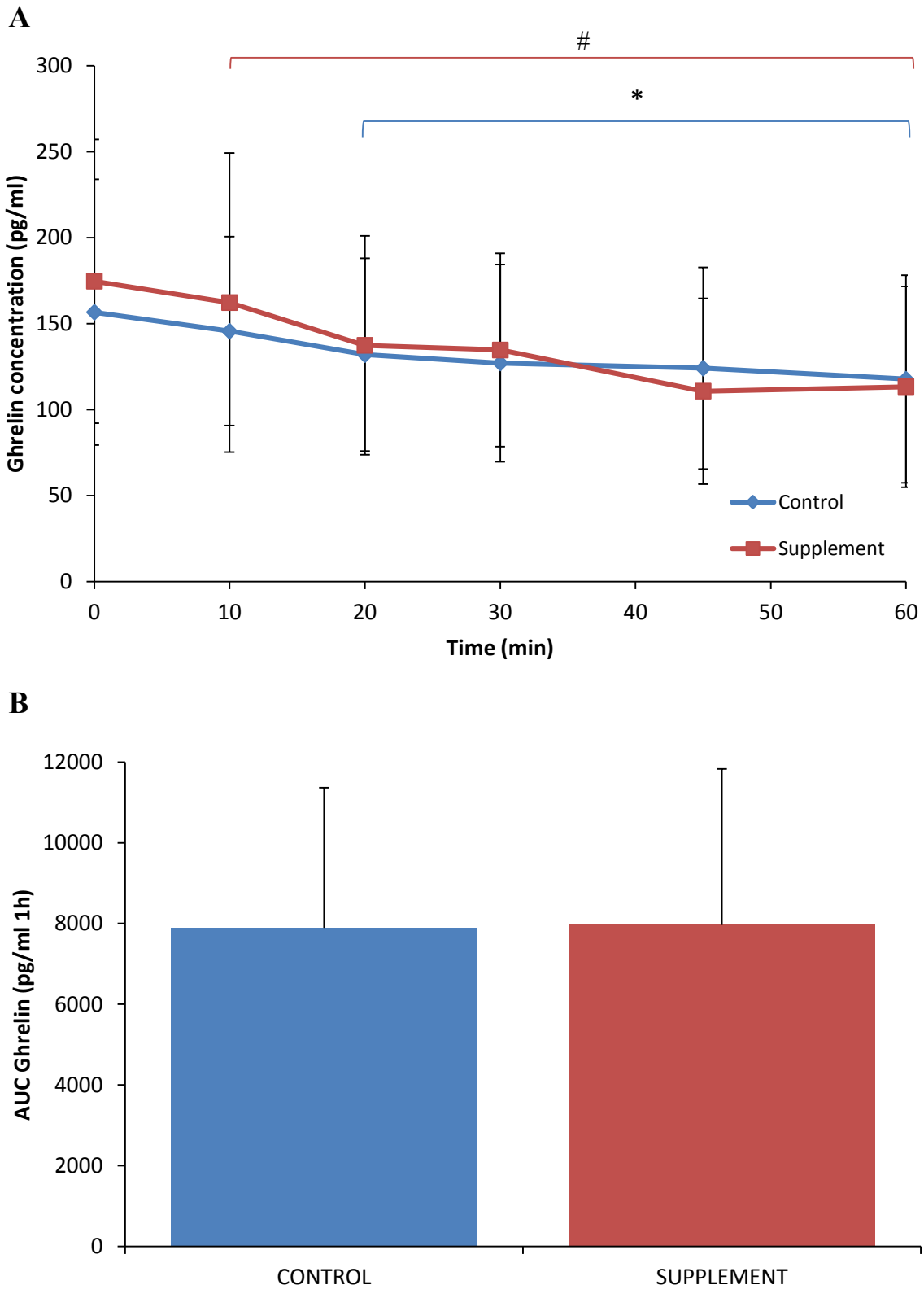

Figure 22. Gut hormone ghrelin (A) Response over 60 min post ingestion of 595 mL 6% fructose solution (B) Area under curve. Treatments were control without fructose supplementation and with 3 d supplementation of 120 g fructose per day. *Significant decrease from 10 min for control trial ($P < 0.05$). #Significant decrease from baseline for supplement trial ($P < 0.01$). Values are mean ± SD (n 10).

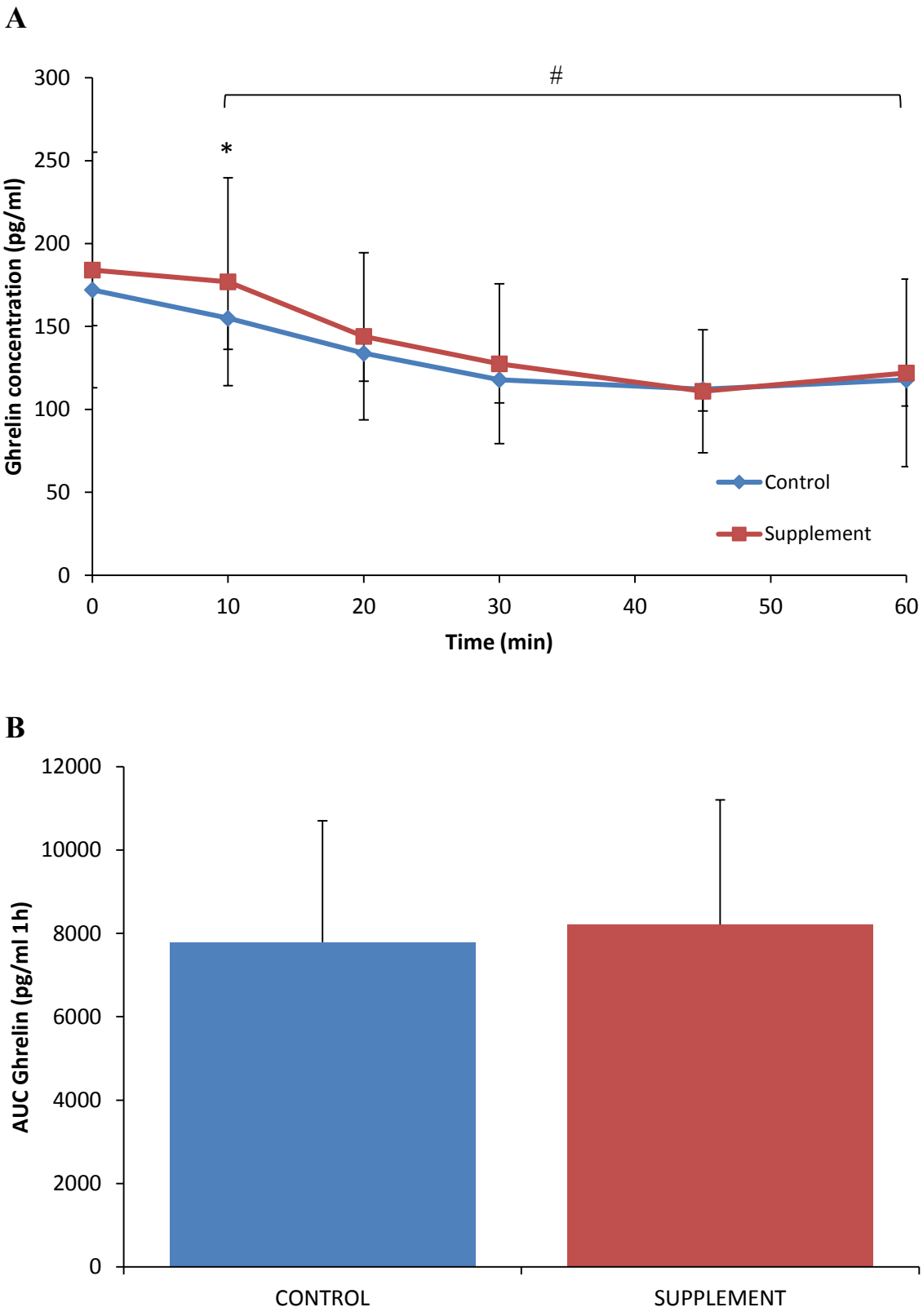

Figure 23. Gut hormone ghrelin (A) Response over 60 min post ingestion of 595 mL 6% glucose solution (B) Area under curve. Treatments were control without fructose supplementation and with 3 d supplementation of 120 g fructose per day. *Significantly greater than control trial ($P < 0.05$). #Significant decrease from baseline for both trials ($P < 0.05$). Values are mean ± SD (n 10).

5.3.3.3. GLP-1

Baseline GLP-1 concentrations were not significantly different between all four trials (FC, 22.09 ± 29.52 pg/mL; FS, 23.81 ± 27.72 pg/mL; GC, 19.83 ± 23.10 pg/mL; GS, 23.68 ± 22.59 pg/mL; $P = 0.947$). Two way ANOVA for all four trials revealed no main effect of trial ($P = 0.881$), a significant effect of time ($P < 0.001$) but no interaction effect ($P = 0.126$). Analysis for fructose ingestion (Figure 26a) showed no main effect of supplementation ($P = 0.685$), a significant effect of time ($P = 0.035$) and no interaction effect ($P = 0.392$). Post-hoc analysis showed GLP-1 concentration increased significantly at 20 min from baseline values then decreased significantly at 60 min during the control trial. No difference in AUC was observed ($P = 0.670$; Figure 26b). Analysis for glucose ingestion (Figure 27a) showed no main effect of supplementation ($P = 0.774$), a significant effect of time ($P < 0.001$) but no interaction effect ($P = 0.857$). Post hoc analysis revealed GLP-1 concentration increased significantly from baseline at 20 min then decreased significantly in the control trial. GLP-1 in the supplement trial however, increased within the first 10 min post ingestion albeit insignificantly, but subsequently decreased significantly to below baseline levels. No difference in AUC was observed ($P = 0.365$; Figure 27b).

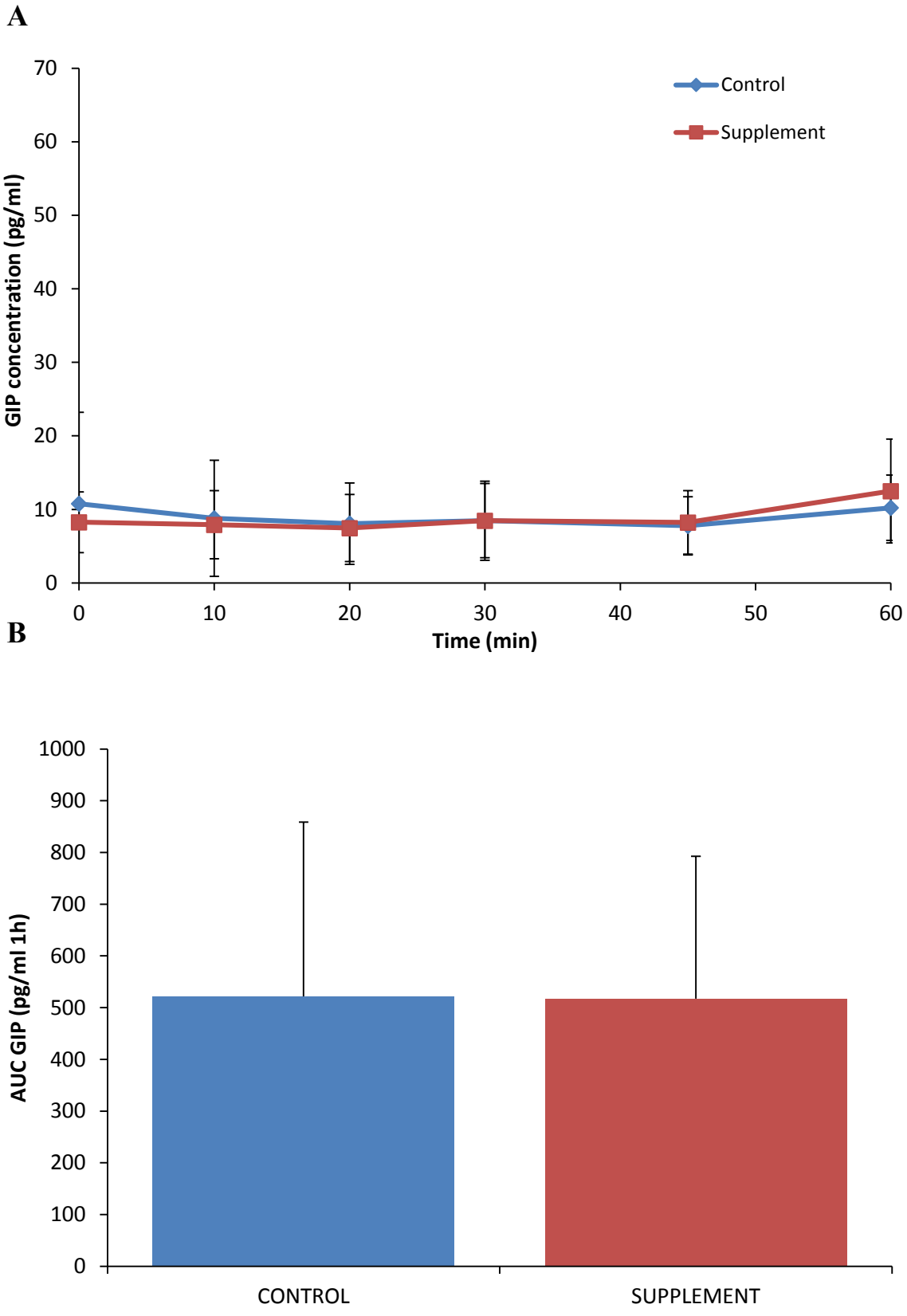

Figure 24. Gut hormone GIP (A) Response over 60 min post ingestion of 595 mL 6% fructose solution (B) Area under curve. Treatments were control without fructose supplementation and with 3 d supplementation of 120 g fructose per day. Values are mean ± SD (*n* 10).

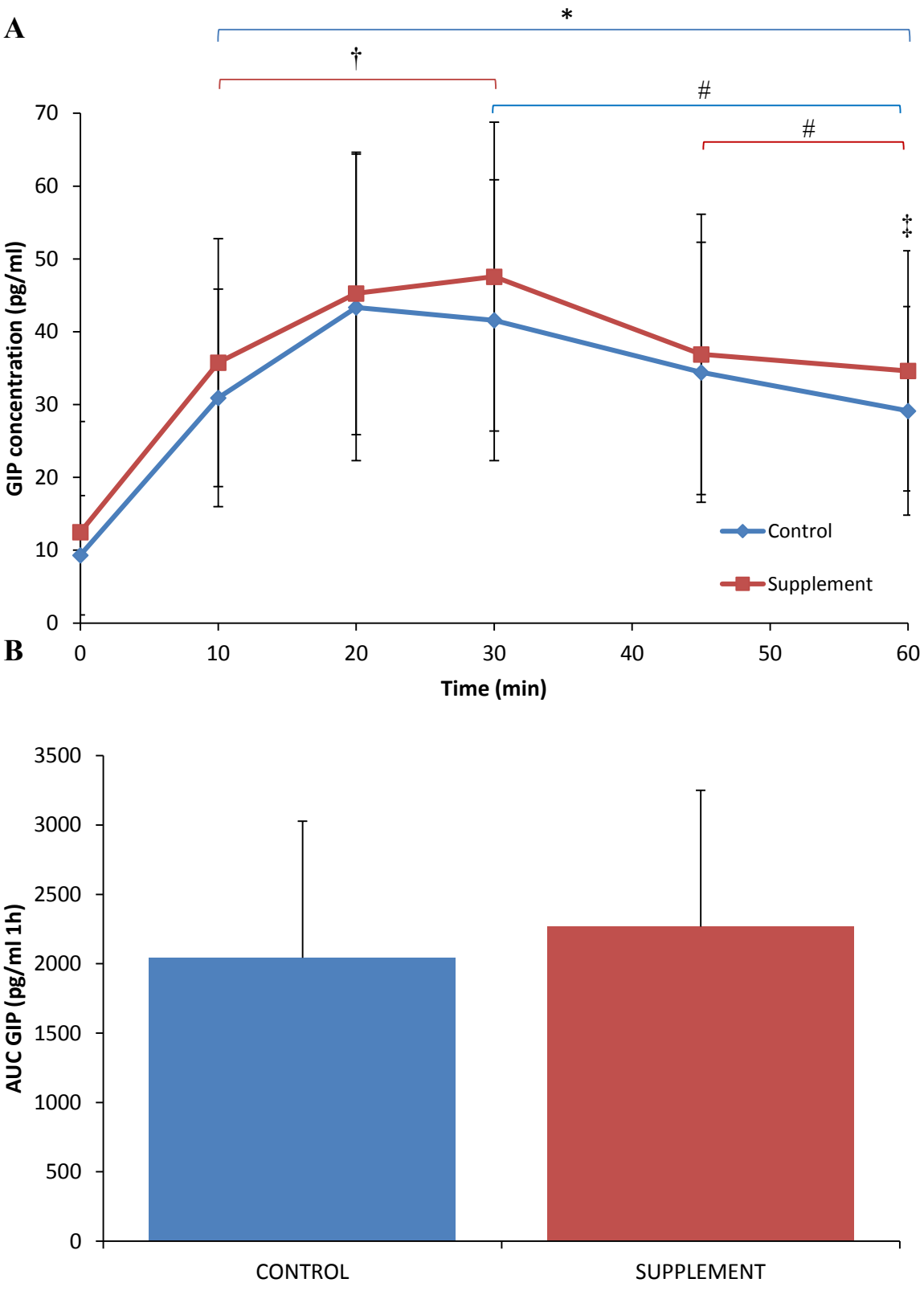

Figure 25. Gut hormone GIP (A) Response over 60 min post ingestion of 595 mL 6% glucose solution (B) Area under curve. Treatments were control without fructose supplementation and with 3 d supplementation of 120 g fructose per day. *Significant increase from baseline for control trial ($P < 0.01$). †Significant increase from baseline for supplement trial ($P < 0.05$). #Significant decrease from 20 min for both trials. ‡Supplement significantly higher than control ($P < 0.05$). Values are mean ± SD (n 10).

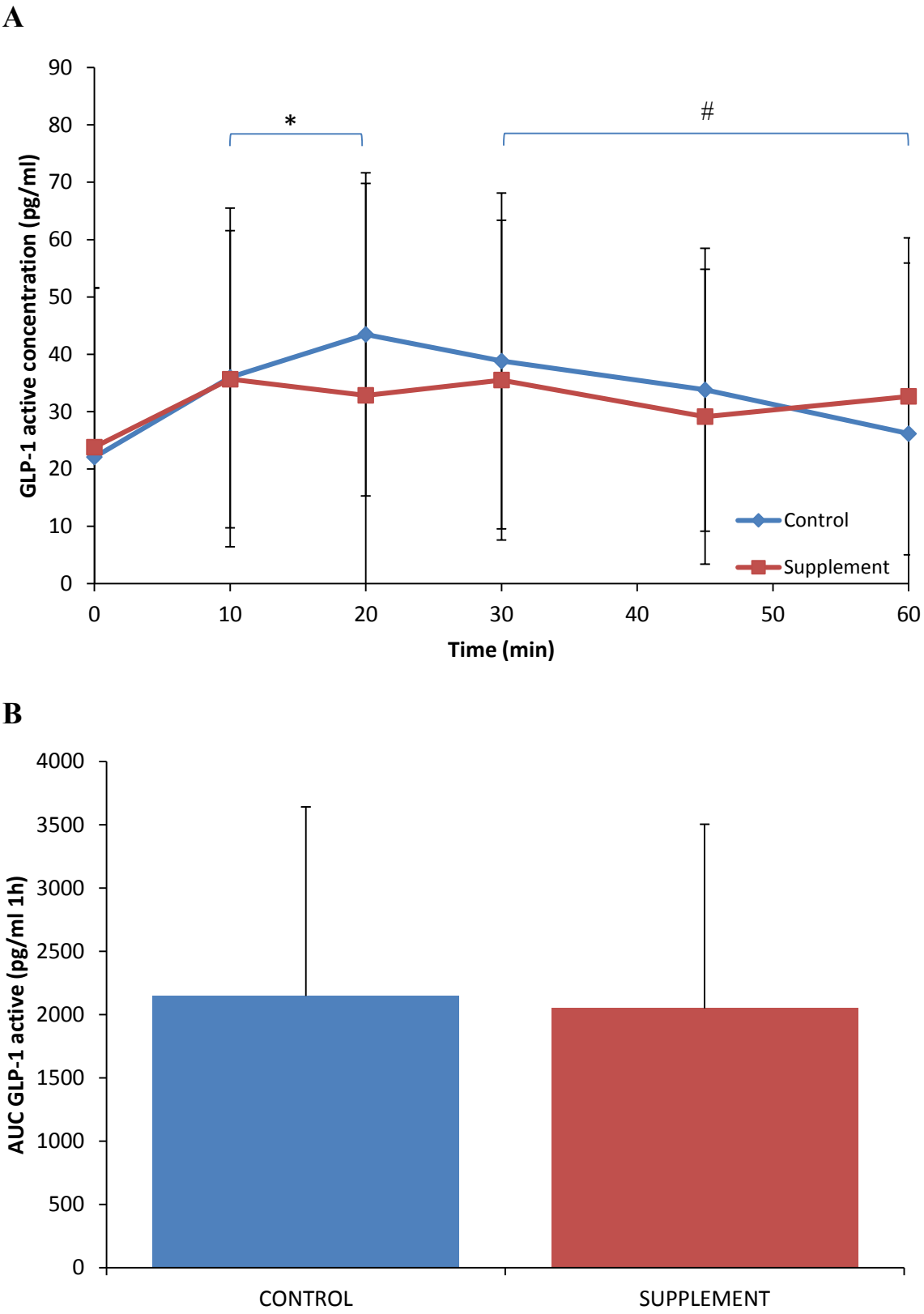

Figure 26. Gut hormone GLP-1 (A) Response over 60 min post ingestion of 595 mL 6% fructose solution (B) Area under curve. Treatments were control without fructose supplementation and with 3 days supplementation of 120 g fructose per day. *Significant increase from baseline for control trial ($P < 0.05$). #Significant decrease from 20 min for control trial ($P < 0.05$). Values are mean ± SD (n 10).

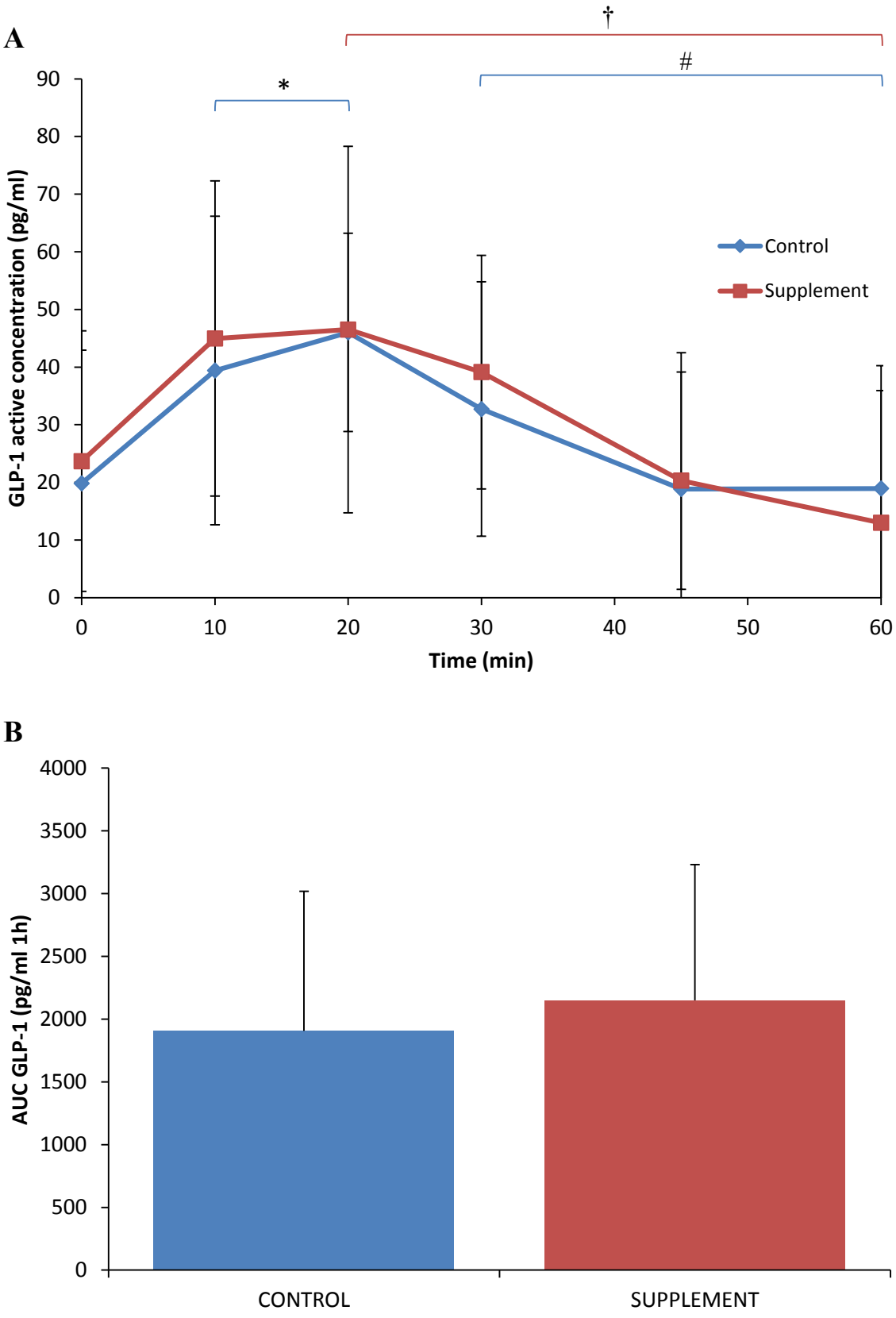

Figure 27. Gut hormone GLP-1 (A) Response over 60 min post ingestion of 595 mL 6% glucose solution (B) Area under curve. Treatments were control without fructose supplementation and with 3 days supplementation of 120 g fructose per day. *Significant increase from baseline for control trial ($P < 0.01$). †Significant decrease from 10 min for supplement trial ($P < 0.05$). #Significant decrease from 20 min for control trial ($P < 0.01$). Values are mean ± SD (n 10).

5.3.3.4. Insulin

Baseline insulin concentrations were not different between all four trials (FC, 483.05 ± 383.64 pg/mL; FS, 396.53 ± 93.16 pg/mL; GC, 396.20 ± 180.37 pg/mL; GS, 425.87 ± 260.60 pg/mL; $P = 0.750$). Two way ANOVA for all four trials showed a main effect of trial ($P < 0.001$), a main effect of time ($P < 0.001$) and an interaction effect ($P < 0.001$). Analysis for fructose ingestion (Figure 28a) showed no main effect of supplementation ($P = 0.341$), a significant effect of time ($P < 0.001$) and no interaction effect ($P = 0.778$). Post-hoc analysis showed a small but significant increase in insulin from baseline levels for both control and supplement trials. No difference in AUC was observed ($P = 0.323$; Figure 28b). Analysis for glucose ingestion (Figure 29a) also showed no main effect of supplementation ($P = 0.975$), an effect of time ($P < 0.001$) and no interaction effect ($P = 0.844$). Post-hoc analysis showed insulin concentrations significantly increased from baseline values at 30 min then significantly decreased thereafter for both trials at 60 min though not back to baseline levels. No difference in AUC was observed ($P = 0.669$; Figure 29b).

5.3.3.5. Leptin

Baseline leptin concentrations were not different between all four trials (FC, 3542.36 ± 2525.04 pg/mL; FS, 3371.53 ± 1934.44 pg/mL; GC, 3857.64 ± 2711.35 pg/mL; GS, 3687.42 ± 2767.98 pg/mL; $P = 0.484$). Two way ANOVA for all four trials showed no effects of trial ($P = 0.352$), time ($P = 0.245$) and interaction ($P = 0.15$). Analysis for fructose ingestion (Figure 30a) showed no effect of supplementation ($P = 0.305$), time ($P = 0.100$) nor interaction ($P = 0.466$). No difference in AUC resulted ($P = 0.381$; Figure 30b). Analysis for glucose ingestion (Figure 31a) also showed the same, no effect of supplementation ($P = 0.934$), time ($P = 0.378$) nor interaction ($P = 0.294$). Again, no difference in AUC resulted ($P = 0.974$; Figure 31b).

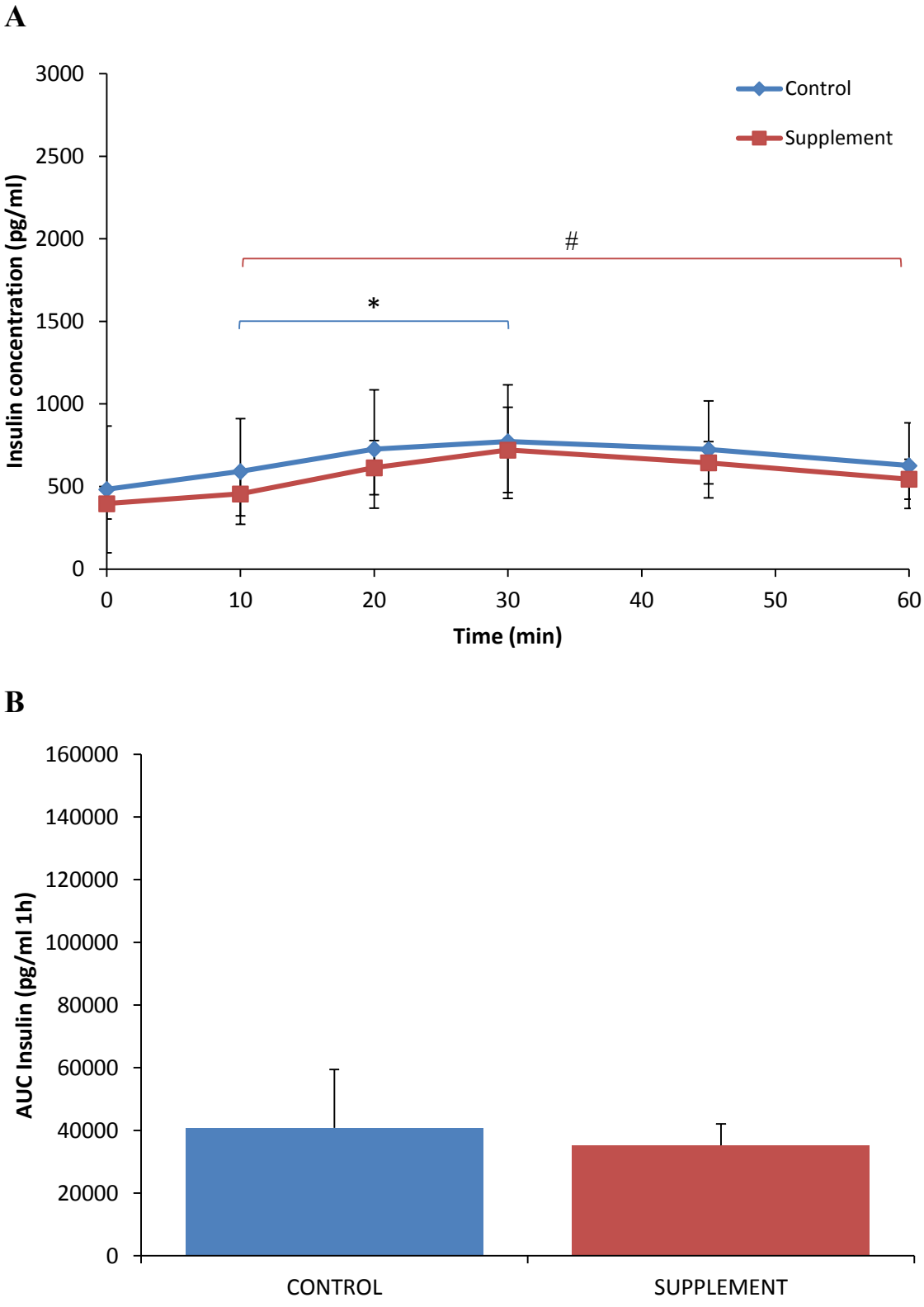

Figure 28. Gut hormone insulin (A) Response over 60 min post ingestion of 595 mL 6% fructose solution (B) Area under curve. Treatments were control without fructose supplementation and with 3 d supplementation of 120 g fructose per day. *Significant increase from baseline for control trial ($P < 0.001$). #Significant increase from baseline for supplement trial ($P < 0.01$). Values are mean ± SD (n 10).

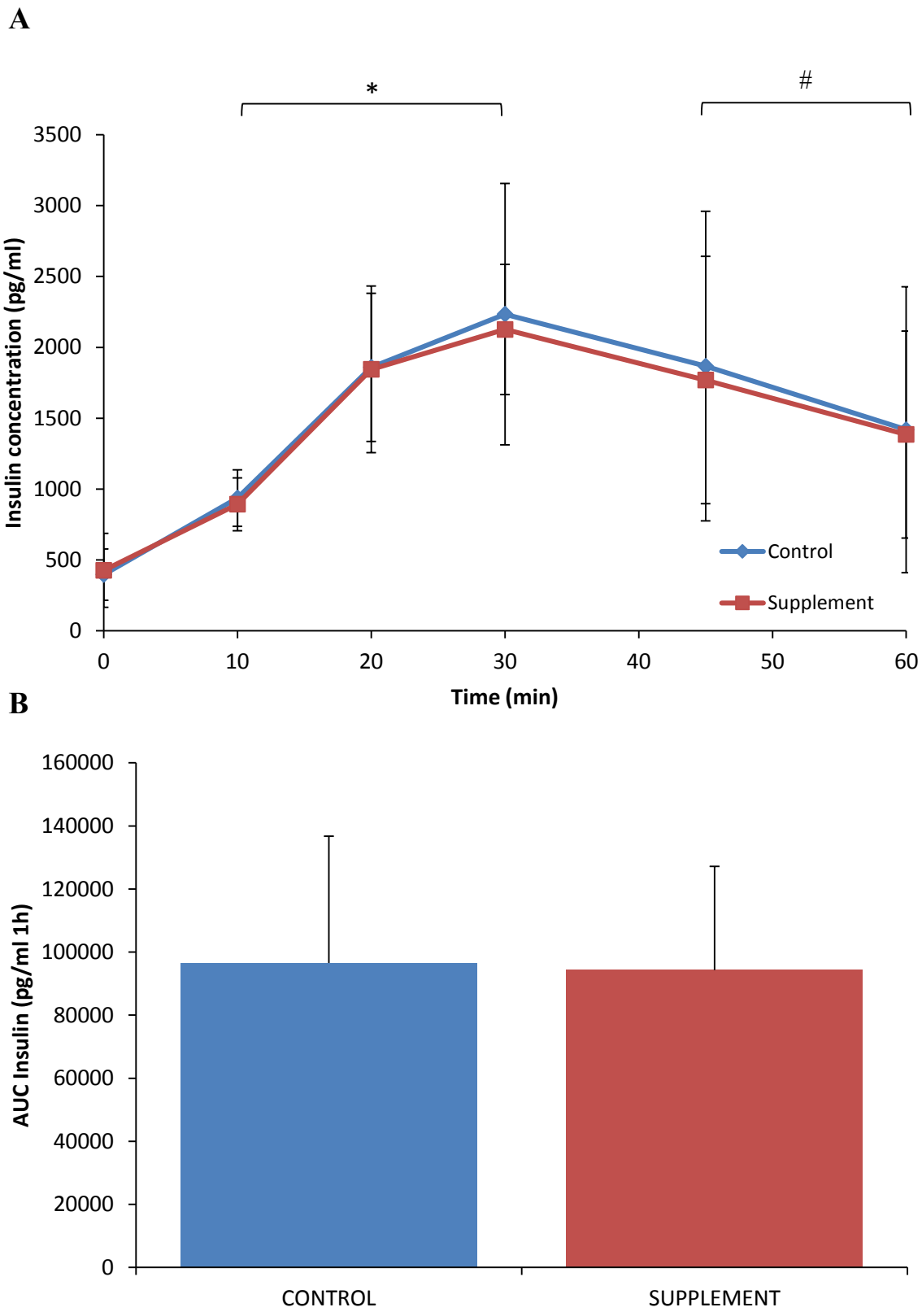

Figure 29. Gut hormone insulin (A) Response over 60 min post ingestion of 595 mL 6% glucose solution (B) Area under curve. Treatments were control without fructose supplementation and with 3 d supplementation of 120 g fructose per day. *Significant increase from baseline for both trials ($P < 0.05$). #Significant decrease from 30 min for both trials ($P < 0.05$). Values are mean ± SD (n 10).

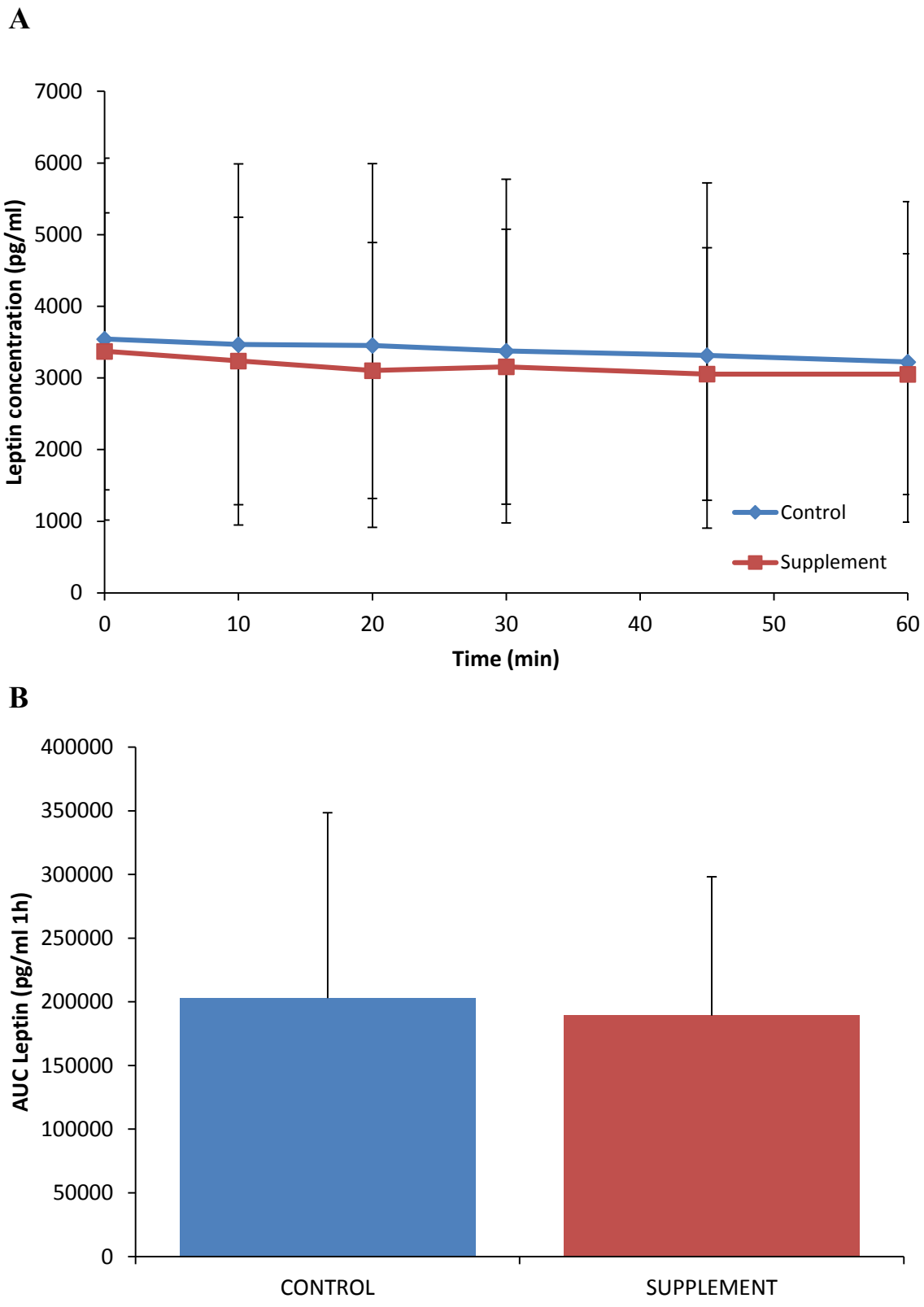

Figure 30. Gut hormone leptin (A) Response over 60 min post ingestion of 595 mL 6% fructose solution (B) Area under curve. Treatments were control without fructose supplementation and with 3 d supplementation of 120 g fructose per day. Values are mean ± SD (*n* 10).

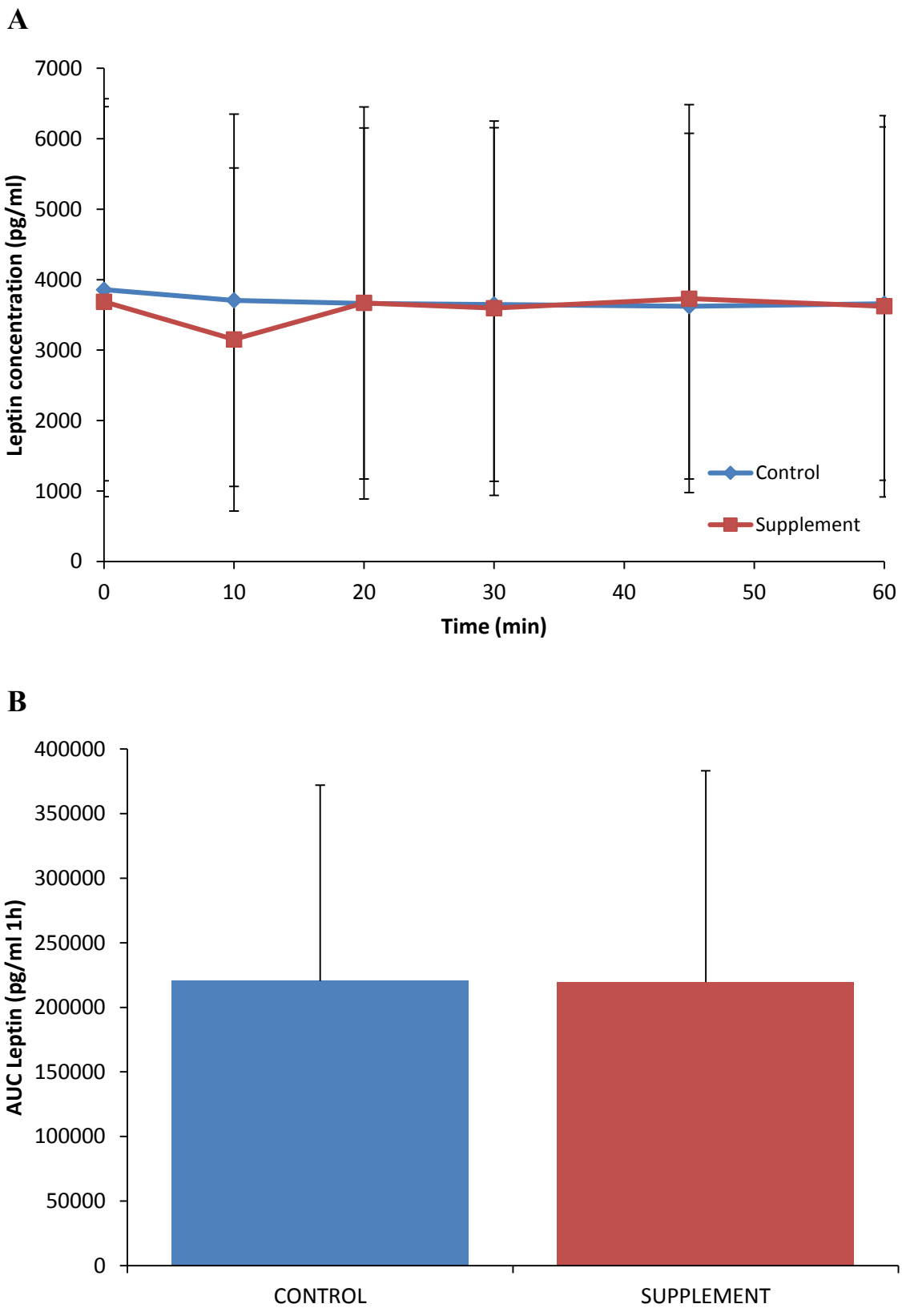

Figure 31. Gut hormone leptin (A) Response over 60 min post ingestion of 595 mL 6% glucose solution (B) Area under curve. Treatments were control without fructose supplementation and with 3 d supplementation of 120 g fructose per day. Values are mean ± SD (*n* 10).

5.3.4. Blood glucose and fructose

Baseline serum glucose concentrations were 5.21 ± 0.46, 5.28 ± 0.30, 5.21 ± 0.40 and 5.11 ± 0.25 mmol/L for FC, FS, GC and GS, respectively ($P = 0.591$). Two way ANOVA for all four trials revealed a significant main effect of trial ($P < 0.001$), a significant effect of time ($P = 0.001$) and an interaction effect ($P < 0.001$). Analysis for fructose ingestion (Figure 32a) revealed no effect of supplementation ($P = 0.880$), a significant effect of time ($P = 0.024$) and no interaction effect ($P = 0.928$). Although changes in concentration were small, post-hoc analysis showed serum glucose concentration significantly increased at 30 min from baseline concentrations then decreased significantly at 60 min for the control trial. Similar response levels over time for the supplement trial were not significantly different ($P = 0.174$). No difference in AUC was observed ($P = 0.955$; Figure 32b). Analysis for glucose ingestion (Figure 33a) showed no effect of supplementation ($P = 0.428$), a significant effect of time ($P < 0.001$) and no interaction effect ($P = 0.658$). Post-hoc analysis revealed serum glucose concentrations significantly increased from baseline, peaking at 30 min, and then decreased significantly to near baseline levels at 60 min for both control and supplementation trials. The rise in concentration at 20 min and peak at 30 min was slightly blunted in the supplementation trial compared to control, but differences were not significant. No difference in AUC existed ($P = 0.502$; Figure 33b).

Baseline serum fructose concentrations were 137.0 ± 48.8, 115.8 ± 39.6, 129.8 ± 36.6 and 139.4 ± 38.4 μmol/L for FC, FS, GC and GS, respectively ($P = 0.163$). Two way ANOVA for all four trials revealed significant trial, time and interactions effects (all $P < 0.001$). Analysis for fructose ingestion (Figure 34a) showed no main effect of supplementation ($P = 0.948$), a significant effect of time ($P < 0.001$) and a significant interaction ($P = 0.011$). Post-hoc analysis revealed serum fructose concentrations increased rapidly and significantly from baseline concentrations within the first 10 min for both control and supplementation trials. Concentrations peaked at 30 min for both trials, with the supplementation trial peak being slightly higher, before both then decreasing slightly by 60 min to similar concentrations. No differences in AUC were seen ($P = 0.588$; Figure 34b). Analysis for glucose ingestion (Figure 35a) showed no main effect of supplementation ($P = 0.547$), no effect of time ($P = 0.172$) but a significant interaction effect ($P = 0.036$). Post-hoc analysis revealed serum fructose concentrations did not change over time in the control trial ($P = 0.645$) but were significantly lower at 45 min compared to baseline ($P = 0.041$) and 20 min ($P = 0.017$) in the supplement trial. No difference in AUC was observed ($P = 0.828$; Figure 35b).

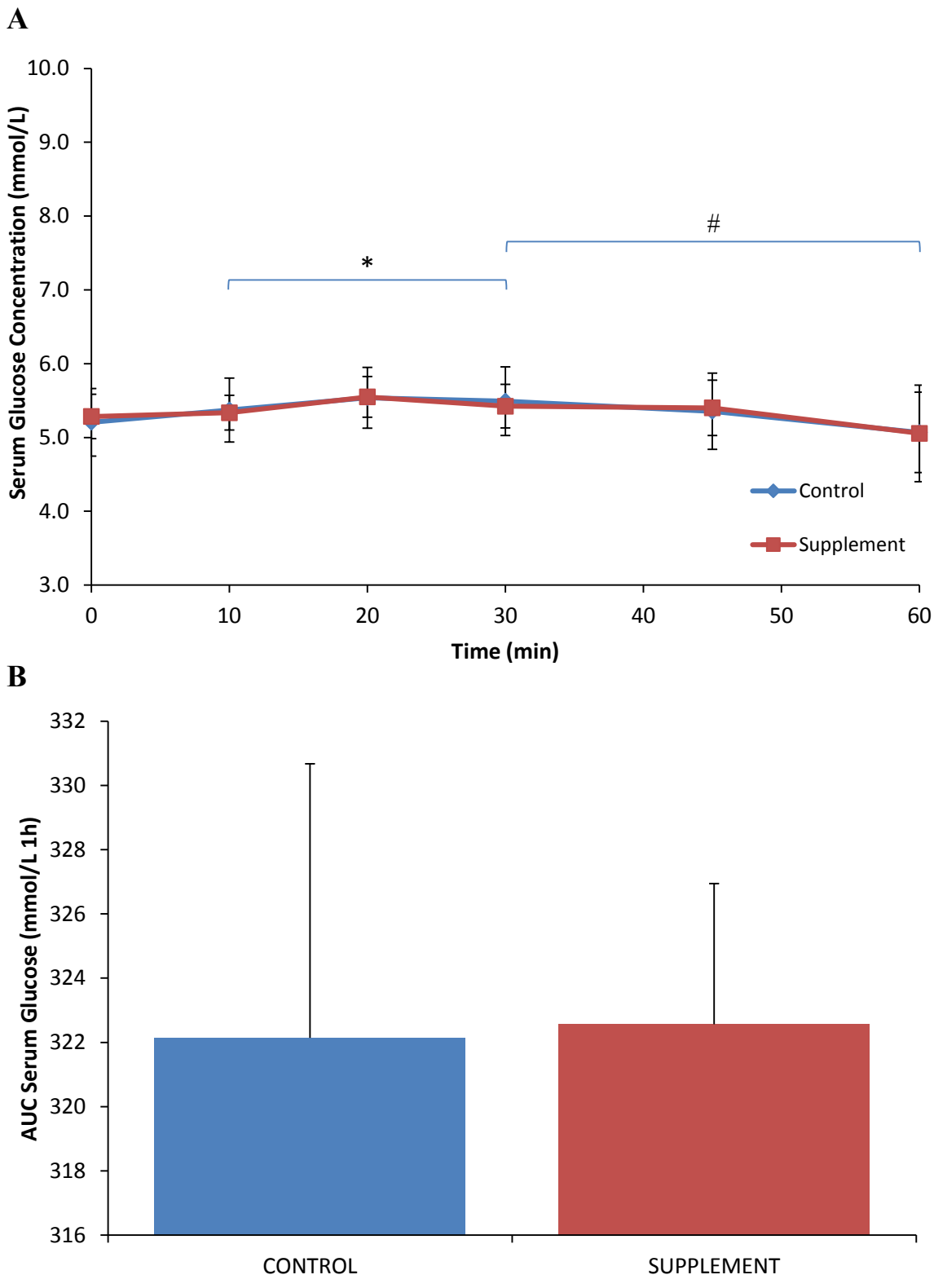

Figure 32. Serum glucose (A) Response over 60 min post ingestion of 595 mL 6% fructose solution (B) Area under curve. Treatments were control without fructose supplementation and with 3 d supplementation of 120 g fructose per day. *Significant increase from baseline for control trial ($P < 0.05$). #Significant decrease from 20 min for control trial ($P < 0.05$). Values are mean ± SD (n 10).

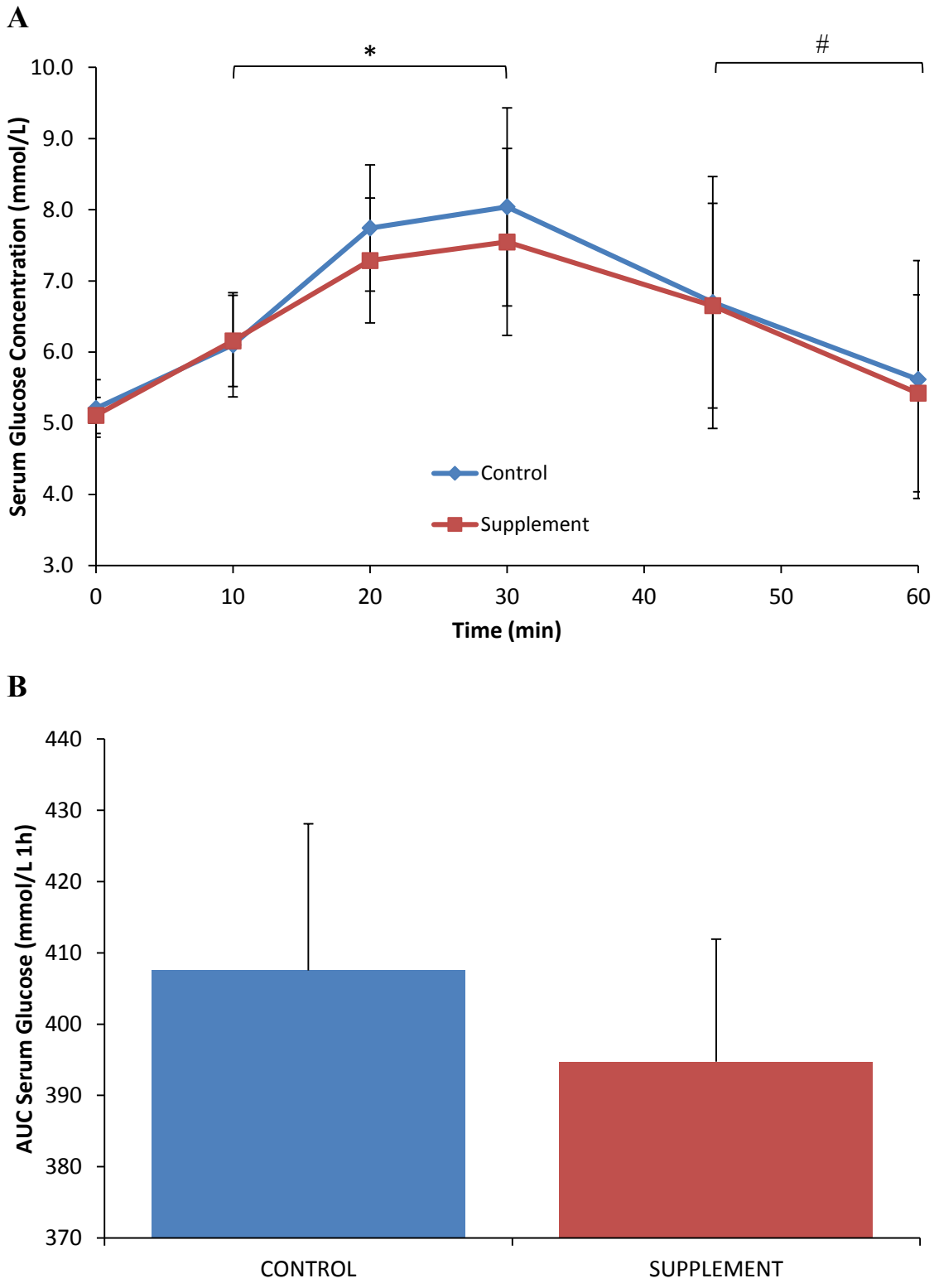

Figure 33. Serum glucose (A) Response over 60 min post ingestion of 595 mL 6% glucose solution (B) Area under curve. Treatments were control without fructose supplementation and with 3 d supplementation of 120 g fructose per day. *Significant increase from baseline for both trials ($P < 0.05$). #Significant decrease from 20 min for both trials ($P < 0.05$). Values are mean ± SD (n 10).

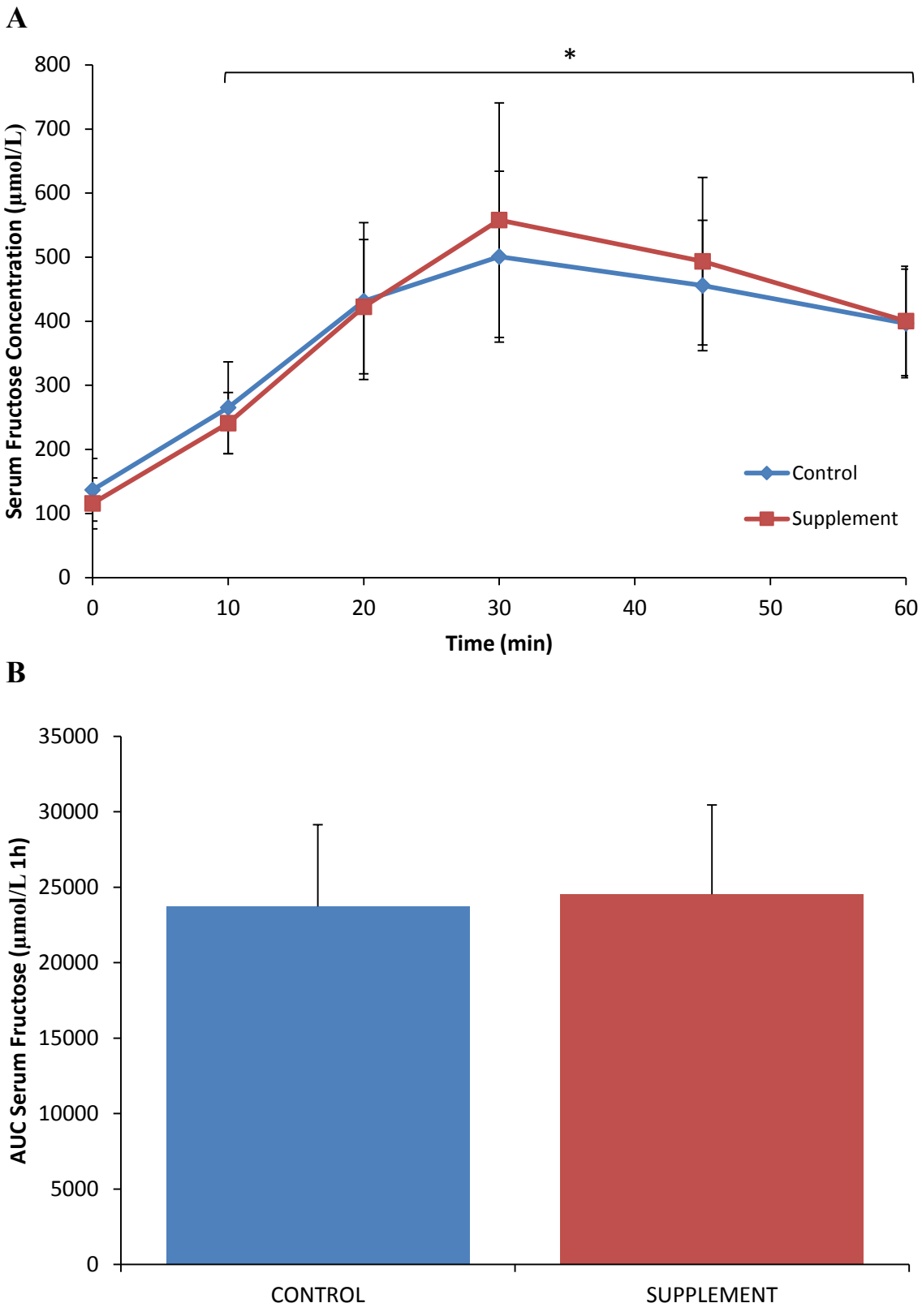

Figure 34. Serum fructose (A) Response over 60 min post ingestion of 595 mL 6% fructose solution (B) Area under curve. Treatments were control without fructose supplementation and with 3 d supplementation of 120 g fructose per day. *Significant increase from baseline for both trials ($P < 0.01$). Values are mean ± SD (n 10).

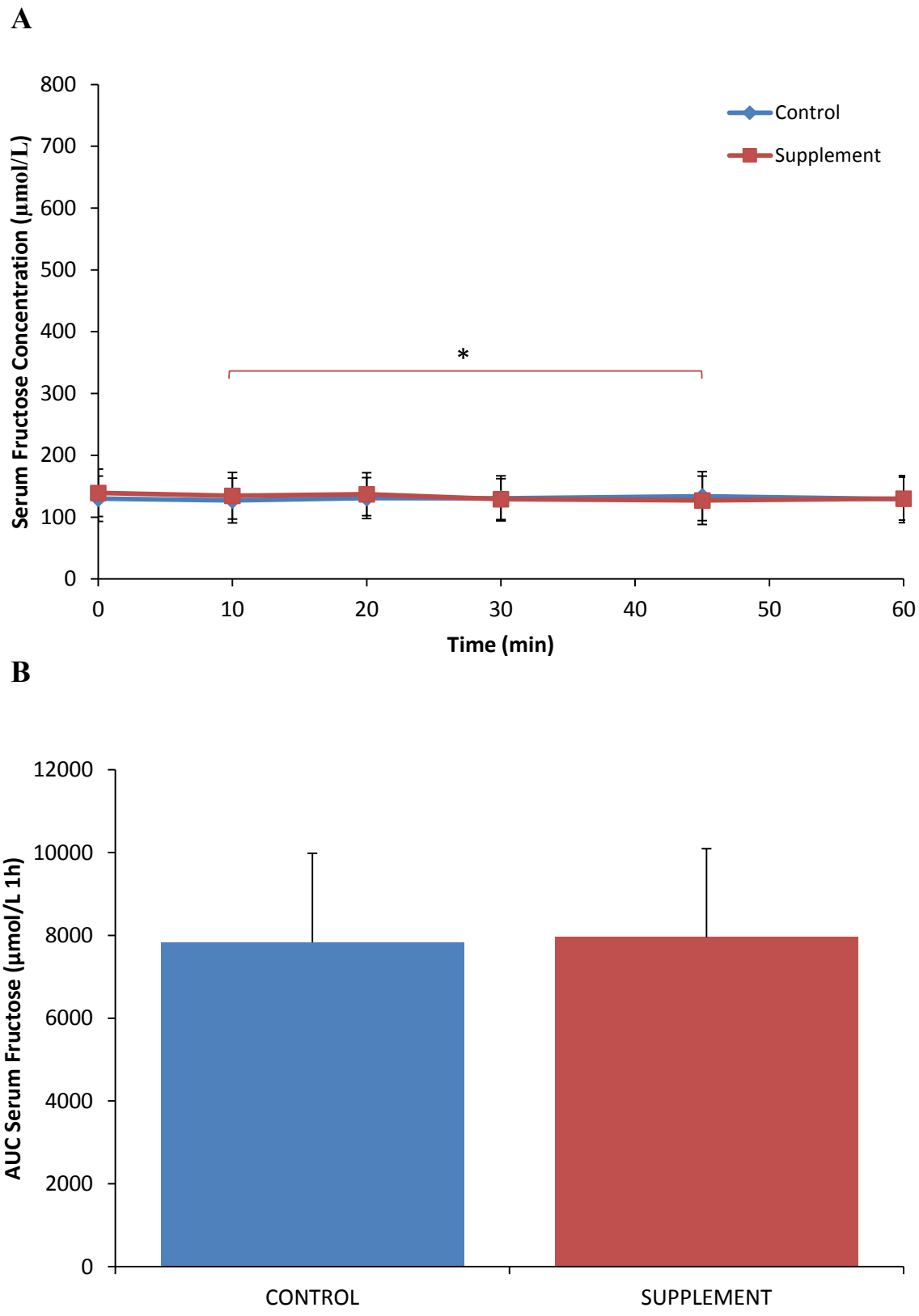

Figure 35. Serum fructose (A) Response over 60 min post ingestion of 595 mL 6% glucose solution (B) Area under curve. Treatments were control without fructose supplementation and with 3 d supplementation of 120 g fructose per day. *Significant decrease from baseline for supplement trial ($P < 0.05$). Values are mean ± SD (n 10).

5.3.5. Serum lactate and triglycerides

Baseline serum lactate concentrations were 1.12 ± 0.41, 1.11 ± 0.30, 1.08 ± 0.39 and 1.00 ± 0.30 mmol/L for FC, FS, GC and GS, respectively ($P = 0.686$). Two way ANOVA for all four trials revealed a significant main effect of trial ($P < 0.001$), a significant effect of time ($P < 0.001$) and an interaction effect ($P < 0.001$). Analysis for fructose ingestion (Figure 36a) revealed no effect of supplementation ($P = 0.511$), a significant effect of time ($P < 0.001$) and no interaction effect ($P = 0.457$). Lactate concentrations increased significantly from baseline values from as early as 10 min for both control and supplementation trials. Mean maximum percent increases were 140% and 124% for FC and FS respectively. No difference in AUC was observed ($P = 0.455$; Figure 36b). Analysis for glucose ingestion (Figure 37a) showed no effect of supplementation ($P = 0.198$), a significant effect of time ($P < 0.001$) and no interaction effect ($P = 0.621$). Lactate concentrations increased significantly from baseline values from 45 min onwards for both trials. Mean maximum percent increases were 44% and 39% for GC and GS respectively. No difference in AUC was observed ($P = 0.208$; Figure 37b).

Baseline triglyceride concentrations were 1.03 ± 0.53, 1.12 ± 0.44, 0.92 ± 0.40 and 1.25 ± 0.45 mmol/L for FC, FS, GC and GS, respectively ($P = 0.082$) with a trend of GS being greater than GC ($P = 0.086$). Two way ANOVA for all four trials revealed no significant main effect of trial ($P = 0.256$), no significant effect of time ($P = 0.695$) but an interaction effect ($P = 0.003$). Analysis for fructose ingestion (Figure 38a) revealed no effect of supplementation ($P = 0.944$), a trend for an effect of time ($P = 0.069$) and no interaction effect ($P = 0.726$). No difference in AUC was seen ($P = 0.448$; Figure 38b). Analysis for glucose ingestion (Figure 39a) showed a significant main effect of supplementation ($P = 0.021$), but no significant effect of time ($P = 0.287$) and no interaction effect ($P = 0.596$). Triglyceride concentration was significantly greater for GS compared to GC at all time points ($P < 0.05$) except at 60 min where it was strongly tending to significance ($P = 0.051$). AUC for GS was also significantly greater than GC ($P = 0.029$; Figure 39b).

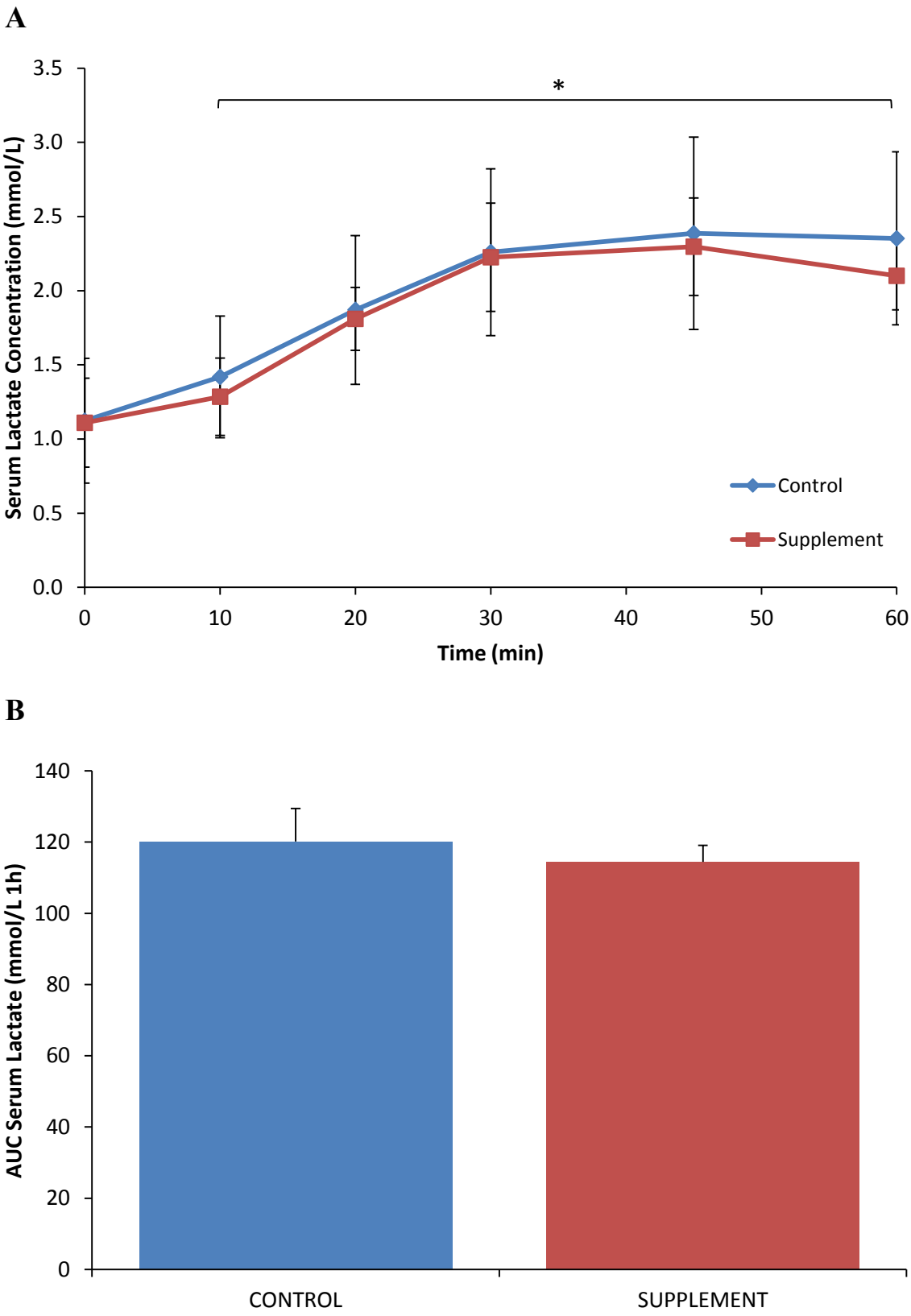

Figure 36. Serum lactate (A) Response over 60 min post ingestion of 595 mL 6% fructose solution (B) Area under curve. Treatments were control without fructose supplementation and with 3 d supplementation of 120 g fructose per day. *Significant increase from baseline for both trials ($P < 0.05$). Values are mean ± SD (n 10).

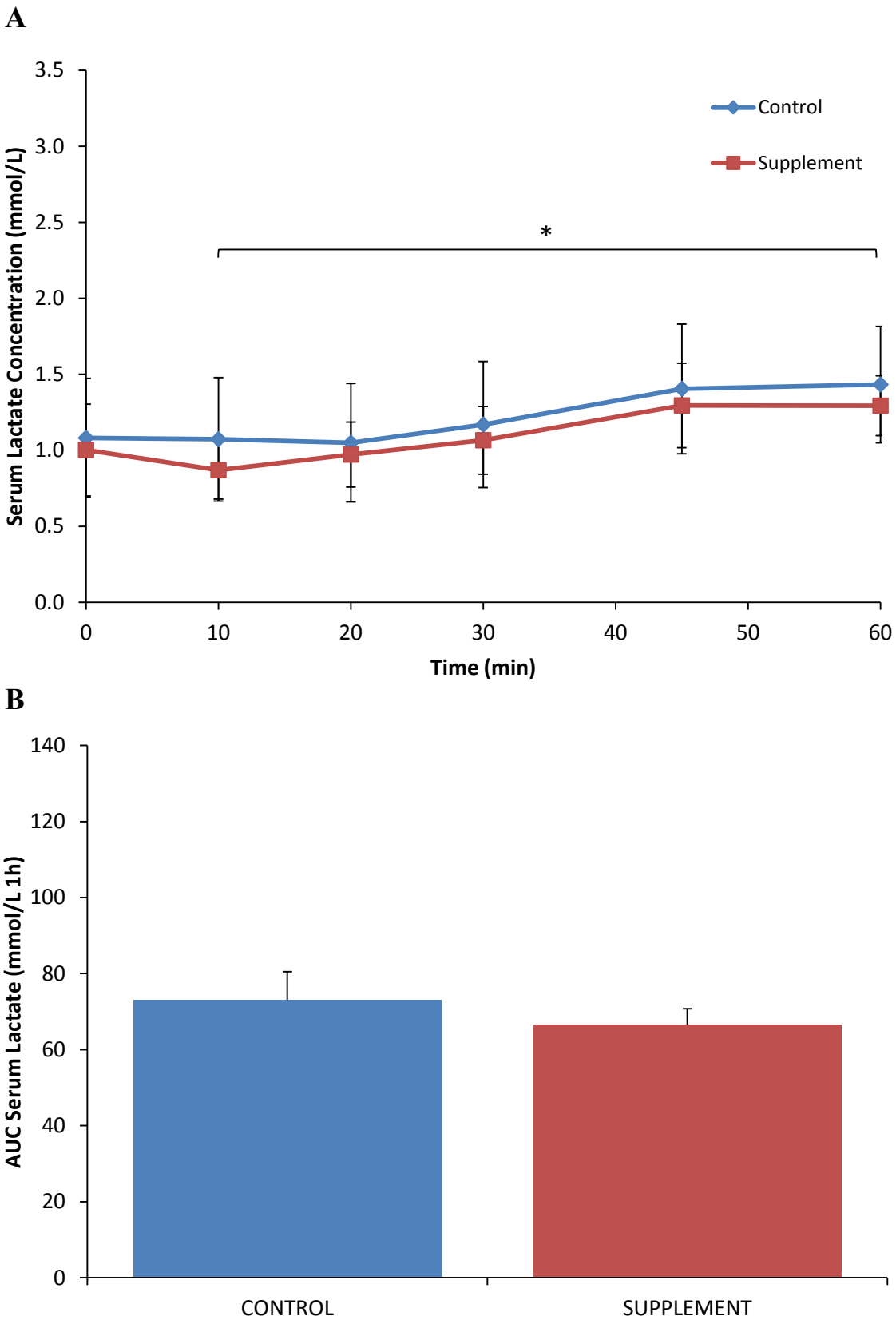

Figure 37. Serum lactate (A) Response over 60 min post ingestion of 595 mL 6% glucose solution (B) Area under curve. Treatments were control without fructose supplementation and with 3 d supplementation of 120 g fructose per day. *Significant increase from baseline for both trials ($P < 0.05$). Values are mean ± SD (n 10).

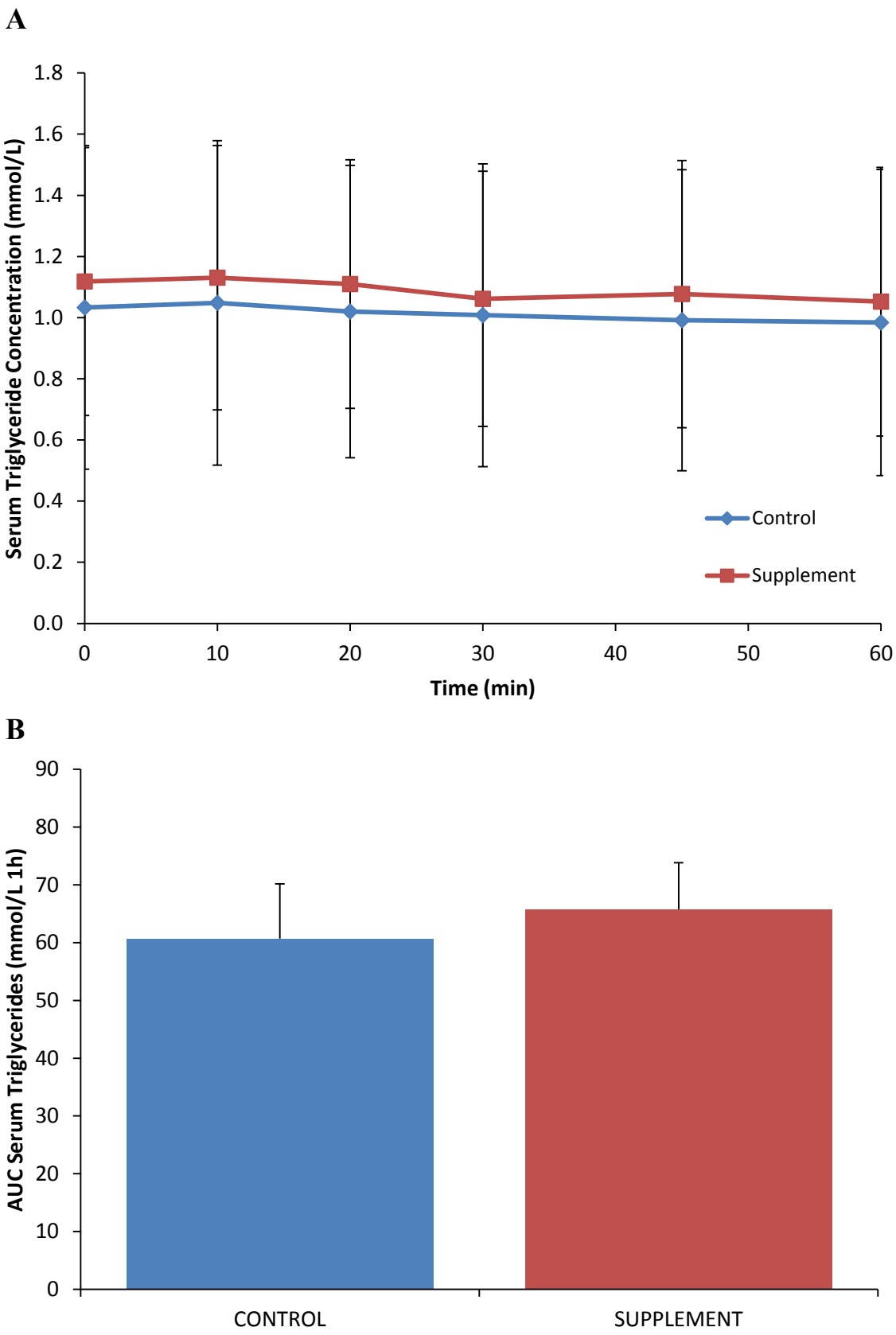

Figure 38. Serum triglycerides (A) Response over 60 min post ingestion of 595 mL 6% fructose solution (B) Area under curve. Treatments were control without fructose supplementation and with 3 d supplementation of 120 g fructose per day. Values are mean ± SD (*n* 10).

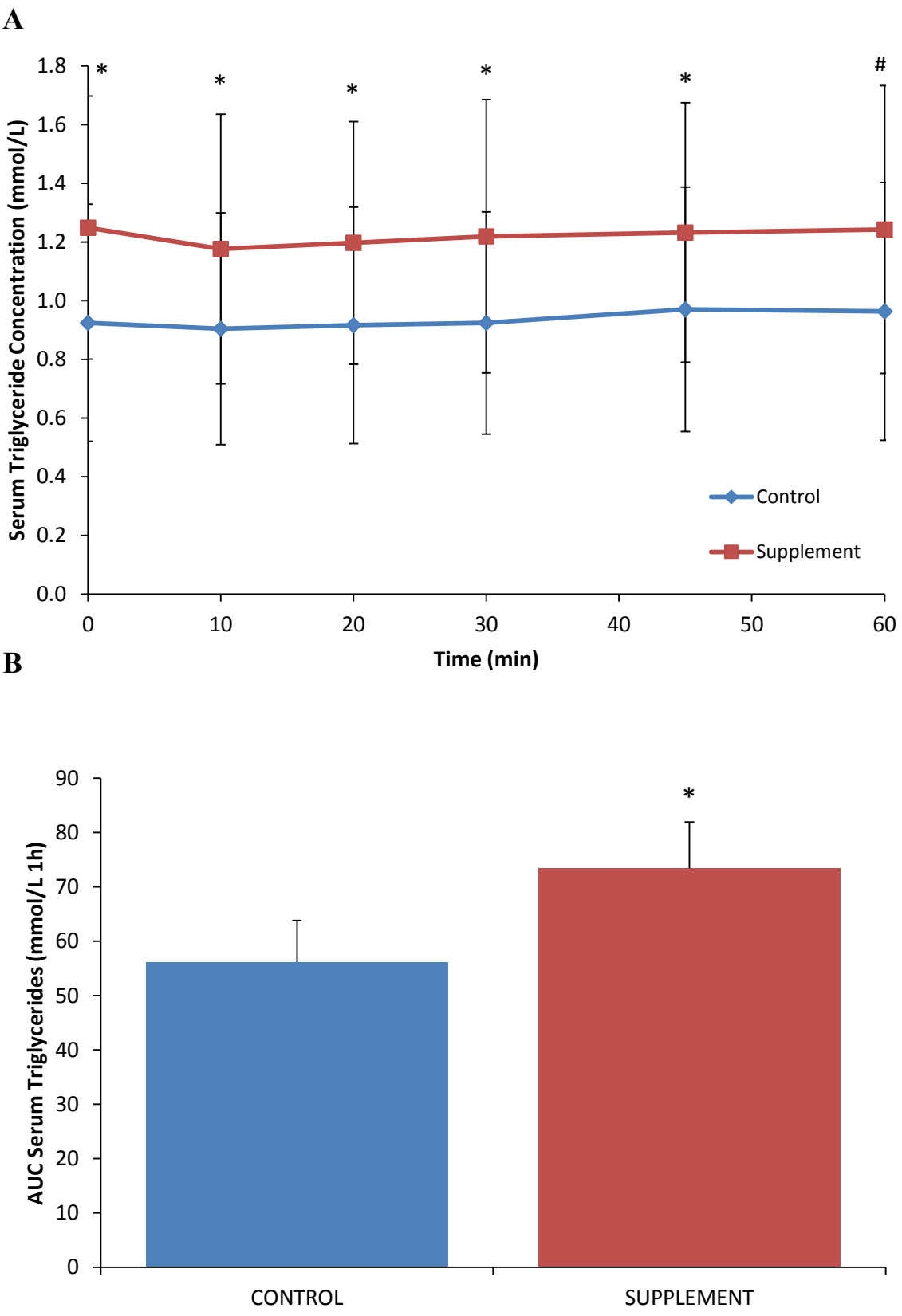

Figure 39. Serum triglycerides (A) Response over 60 min post ingestion of 595 mL 6% glucose solution (B) Area under curve. Treatments were control without fructose supplementation and with 3 d supplementation of 120 g fructose per day. *Significantly higher than control trial ($P < 0.05$). #Trend higher than control ($P = 0.51$). Values are mean ± SD (n 10).

5.3.6. Appetite and satiety

5.3.6.1. Fructose ingestion

Subjective feeling of hunger for fructose ingestion (Figure 40) showed a trend of supplement effect (P = 0.09) with a slight suppression of hunger for the supplement trial compared to control from 10 min to 40 min. No main effect of time (P = 0.106) nor interaction (P = 0.477) was present, however. Complementary to the trend in a main effect of supplementation, AUC for hunger also tended to be lower for supplement compared to control (FC, 4032 ± 1365 mm^2 vs. FS, 3569 ± 1733 mm^2; P = 0.095).

Feeling of fullness remained relatively low and unchanged throughout the 60 min period though a more gradual decrease over time after an initial slight increase can be seen for the control trial (Figure 41). No effect of supplementation (P = 0.231), time (P = 0.144) or interaction (P = 0.236) was found. No difference in AUC was also seen (FC, 1015 ± 953 mm^2 vs. FS, 1213 ± 1028 mm^2; P = 0.155).

Differences were seen with ratings of prospective food consumption, however (Figure 42). A main effect of supplementation (P = 0.027) was evident with no main effect of time (P = 0.101) and interaction (P = 0.205). Post-hoc analysis revealed ratings were temporarily significantly lower for FS compared with FC from 30 to 50 min. This resulted in a significantly lower AUC than control (FC, 4022 ± 1325 mm^2 vs. FS, 3768 ± 1498 mm^2; P = 0.02).

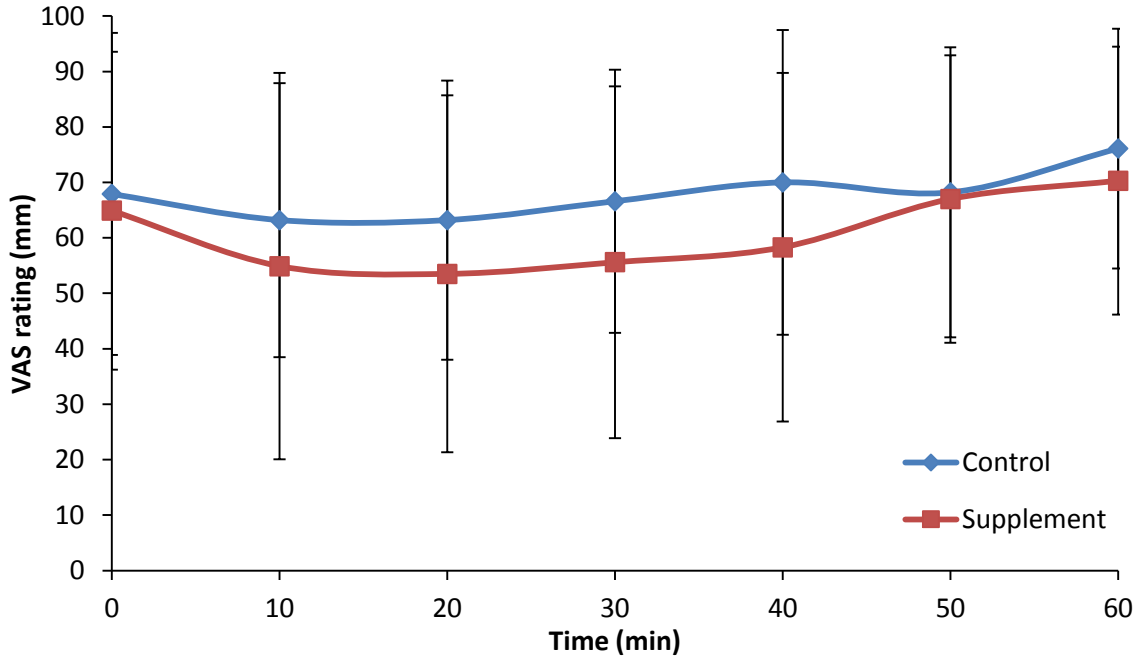

Figure 40. Subjective feeling of hunger (A) Response over 60 min post ingestion of 595 mL 6% fructose solution (B) Area under curve. Treatments were control without fructose supplementation and with 3 d supplementation of 120 g fructose per day. Values are mean ± SD (n 10).

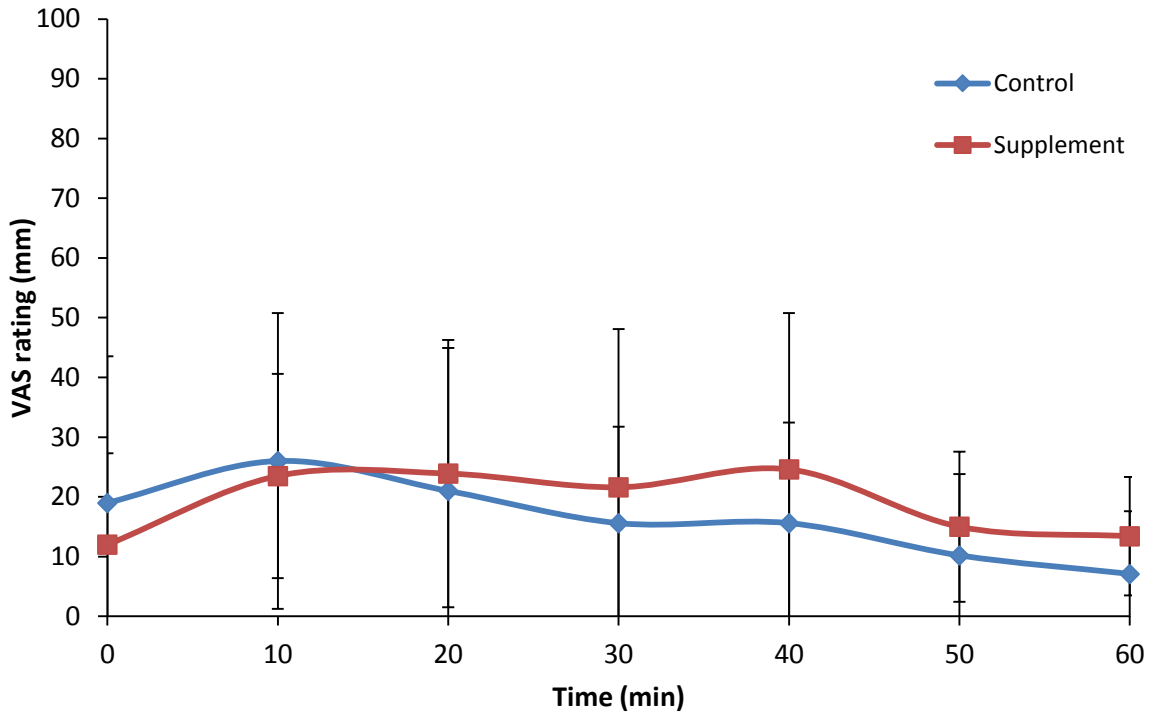

Figure 41. Subjective feeling of fullness (A) Response over 60 min post ingestion of 595 mL 6% fructose solution (B) Area under curve. Treatments were control without fructose supplementation and with 3 d supplementation of 120 g fructose per day. Values are mean ± SD (*n* 10).

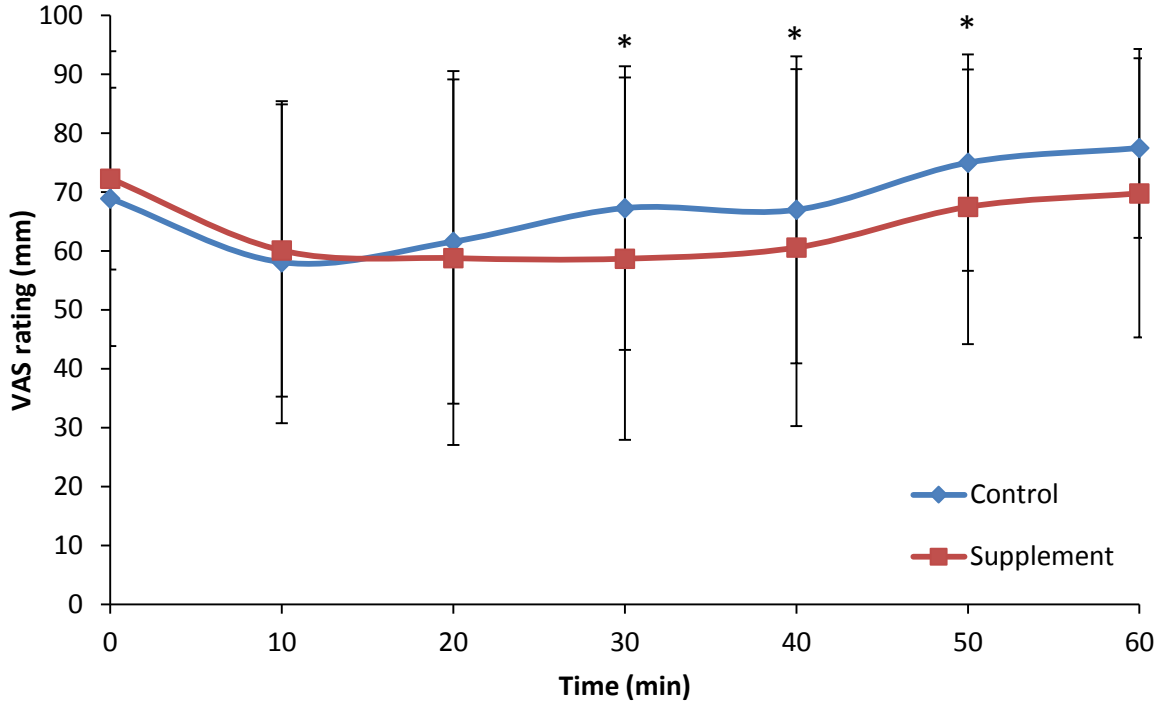

Figure 42. Subjective feeling of prospective food consumption (A) Response over 60 min post ingestion of 595 mL 6% fructose solution (B) Area under curve. Treatments were control without fructose supplementation and with 3 d supplementation of 120 g fructose per day. *Significantly different at time-point ($P < 0.05$). Values are mean ± SD (*n* 10).

5.3.6.2. Glucose ingestion

Subjective feeling of hunger for glucose ingestion was relatively unchanged over the test duration although a drift of increased hunger in the latter half of the hour can be seen for the supplementation trial (Figure 43). No main effect of supplementation ($P = 0.231$), time ($P = 0.410$) or interaction ($P = 0.237$) was found. No difference in AUC was also observed ($P = 0.466$).

A slight increase in fullness was seen in the first 10 min following drink ingestion after which a gradual decrease back to fasted ratings resulted (Figure 44). A trend of a main effect of supplementation was shown for feeling of fullness ($P = 0.083$). No main effect of time ($P = 0.235$) nor interaction ($P = 0.523$) was seen, however. There was also no difference in AUC ($P = 0.107$).

Prospective food consumption also did not change much during the trials (Figure 45). No effect of supplementation ($P = 0.550$), time ($P = 0.370$) or interaction ($P = 0.661$) was observed. No difference in AUC was also seen ($P = 0.753$).

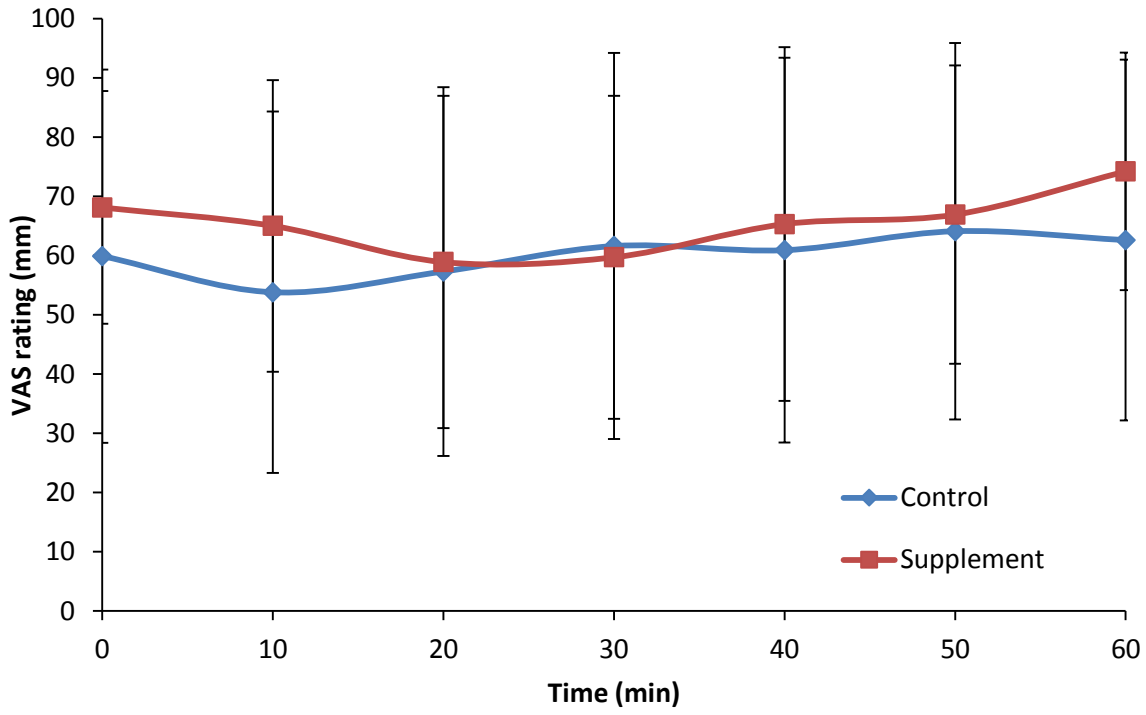

Figure 43. Subjective feeling of hunger (A) Response over 60 min post ingestion of 595 mL 6% glucose solution (B) Area under curve. Treatments were control without fructose supplementation and with 3 d supplementation of 120 g fructose per day. Values are mean ± SD (*n* 10).

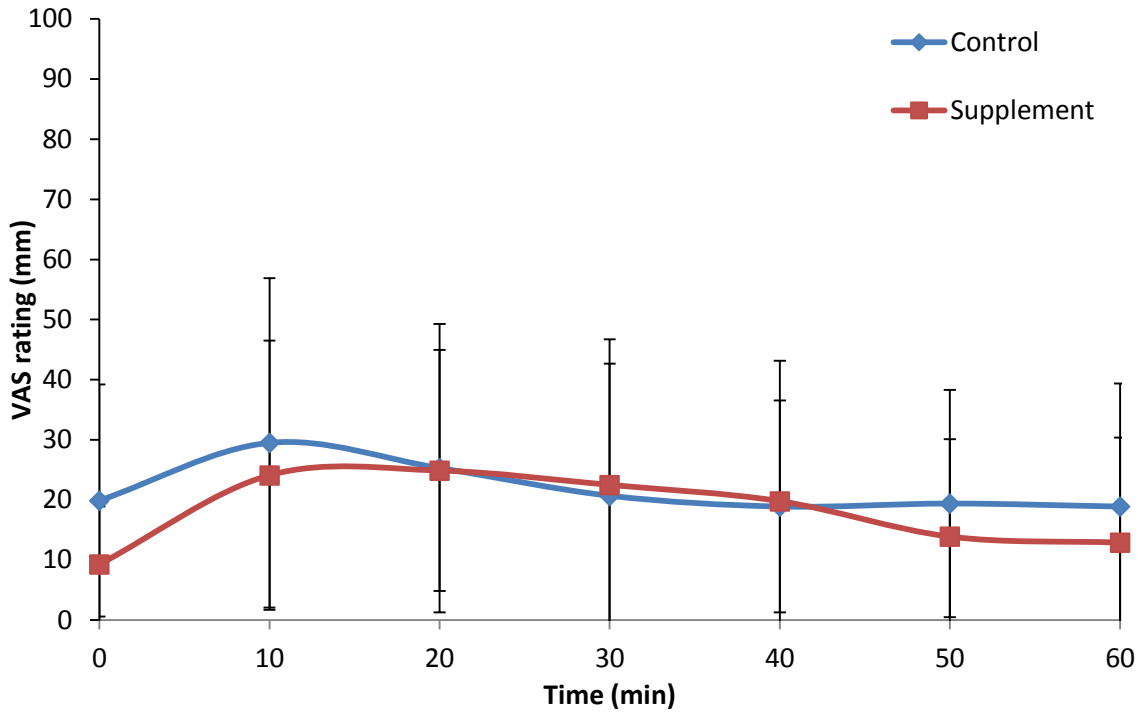

Figure 44. Subjective feeling of fullness (A) Response over 60 min post ingestion of 595 mL 6% glucose solution (B) Area under curve. Treatments were control without fructose supplementation and with 3 d supplementation of 120 g fructose per day. Values are mean ± SD (*n* 10).

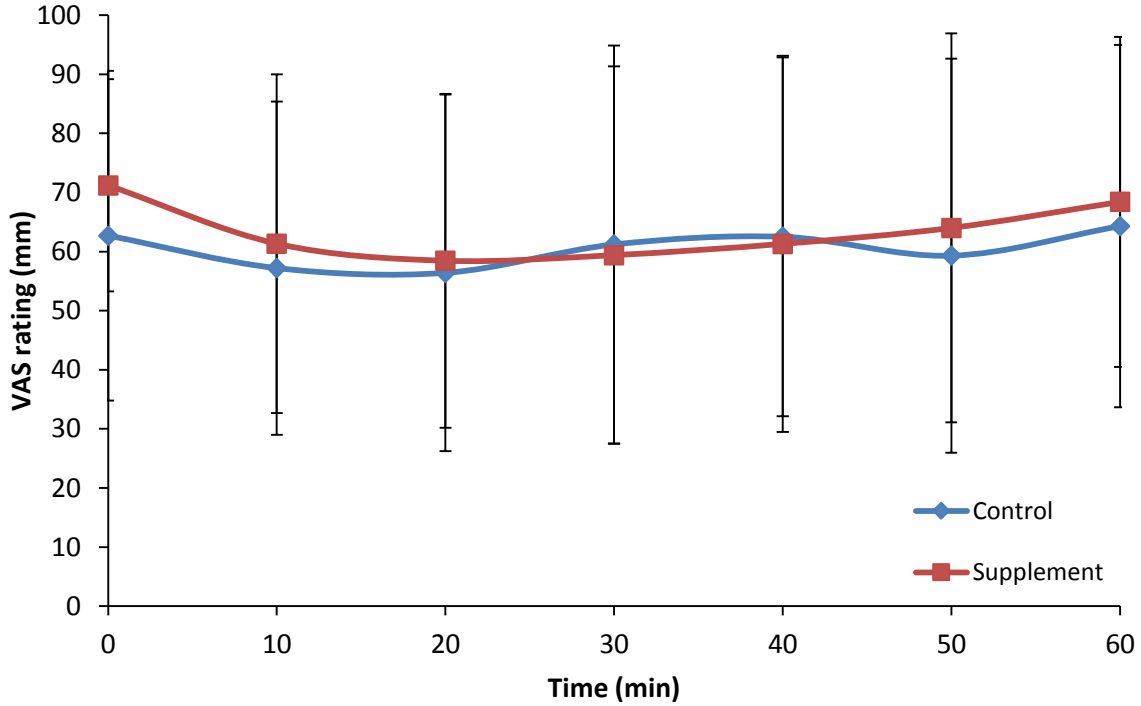

Figure 45. Subjective feeling of prospective food consumption (A) Response over 60 min post ingestion of 595 mL 6% glucose solution (B) Area under curve. Treatments were control without fructose supplementation and with 3 d supplementation of 120 g fructose per. Values are mean ± SD (*n* 10).

5.4. DISCUSSION

The gastric emptying results of this study are in concordance with that of previous observations in chapter 4 and strengthen the reliability of the data. Once again a monosaccharide specific adaptation in gastric emptying rate was shown following short-term dietary supplementation of fructose. Gastric emptying of a fructose solution was accelerated whilst emptying of a glucose solution was unchanged. These results may be explained by subtle changes and differences in gut hormone responses seen in this present study.

Supplementation of the diet with fructose resulted in a delay in the postprandial suppression of ghrelin following the ingestion of fructose and a greater ghrelin concentration at 10 min with the ingestion of glucose. Furthermore, fasting ghrelin levels were slightly elevated by 7-11% after three days of supplementation. This is in agreement relatively with the results of Lindqvist *et al*. (2008) who reported a 40% increase in fasting ghrelin concentrations following two weeks high fructose diet in rats. As ghrelin has been shown to promote gastric emptying rate (Levin, Edholm, Schmidt, Gryback, Jacobsson Degerblad *et al*., 2006; Murray, Martin, Patterson, Taylor, Ghatei & Kamm *et al*., 2005; Asakawa, Inui, Kaga, Yuzuriha, Nagata, Ueno *et al*., 2001), both of the former postprandial observations would suggest a slight initial acceleration of emptying for both fructose and glucose ingestion. Hence, this does not explain the specific acceleration of fructose emptying. However, the differences in the other hormone responses to counter the changes in ghrelin response may. One potential explanation is that there was no difference in GIP response for fructose ingestion whilst there was a trend for significantly greater GIP response for glucose ingestion following supplementation. This difference in supplementation effect may have been due to the fact that GIP secretion is comparatively limited in response to fructose ingestion (chapter 3). These results contrast those of Horowitz *et al*. (1996) who showed GIP response increased for both glucose and fructose ingestion following dietary glucose supplementation. However, whether these GIP results in the present study indicate a potential mechanism for the specific acceleration of fructose but not glucose emptying is questionable as the influence of GIP on gastric emptying rate is unclear with mixed results. Pharmacological doses of GIP in healthy men have been shown to have no effect on gastric emptying rate (Meier, Goetze, Anstipp, Hagemann, Holst, Schmidt *et al*., 2004) as well as moderately accelerate emptying (Edholm, Degerblad, Gryback, Hilsted, Holst, Jacobsson *et al*., 2010). It may therefore be the differences in GLP-1 response observed that hold the key to the present emptying results. The ingestion of fructose following supplementation resulted in the attenuation of GLP-1

secretion compared to the control trial. GLP-1 levels significantly increased then decreased in the fructose control trial whilst no significant changes over time was seen for the supplemented trial. As GLP-1 is known to strongly inhibit gastric emptying and has been termed as an 'ileal brake' (Wishart, Horowitz, Morris, Jones & Nauck, 1998; Wettergren, Schjoldager, Mortensen, Myhre, Christiansen & Holst, 1993), this would suggest a reduced ileal brake effect and a resultant faster emptying. This was not seen with the ingestion of glucose however. Postprandial GLP-1 response similarly increased then decreased following glucose ingestion and in fact was slightly higher during the supplemented trial. Thus taken altogether, it seems that attenuation in GLP-1 secretion combined with an attenuation of ghrelin suppression may be responsible for the accelerated emptying of a fructose solution. On the other hand, a slightly greater GLP-1 response and/or a greater GIP or possibly other gut hormones not measured in this study likely countered the attenuation of ghrelin suppression to result in no significant changes in gastric emptying of a glucose solution.

It has previously been shown that changes in CCK and ghrelin concentrations following high protein or high fat diets have been associated with complementary changes in mRNA levels (Lee *et al*., 2002; Liddle, Morita, Conrad & Williams, 1988). It is unknown whether the changes in serum concentrations of gut hormones in this present study were simply changes in hormone release or whether the three days of increased dietary fructose load led to up- or down-regulation of genes and associated changes in mRNA levels. This should be investigated further. As for the mechanism of altered hormone release and intestinal feedback, changes in sensitivity or stimulation to the presence of fructose may have occurred as a result of the increased fructose consumption. This may have been through increased expression of gut sweet taste receptor T1R2/T1R3 which has been detected in the intestinal tract and enteroendocrine cells (Bezencon, le Coutre & Damak, 2007; Dyer, Salmon, Zibrik & Shirazi-Beechy, 2005) and has been shown to be involved in the secretion of gut hormones including GLP-1 and PYY (Gerspach, Steinert, Schonenberger, Graber-Maier & Beglinger, 2011). Alternatively, as discussed in chapter 4, enhanced absorption as a result of GLUT5 upregulation and consequently greater transporter activity may be involved in the mediation of gut hormone release.

Three days of fructose supplementation did not result in a change in leptin concentration in this study. This is most likely due to the fact that no change in BM, and thus assumed no change in body fat/adiposity, occurred over this short supplementation period where only an extra 1440 kcal was consumed over the three days. This result is in contrast to the results of Le *et al*. (2006) who reported a significant increase in leptin levels

within one week of a high fructose diet. The longer supplementation period with an approximate mean extra 2898 kcal consumption may have accounted for this difference though the authors of that study also reported no change in body weight and body fat percentage, however.

The rate of gastric emptying is logically theorised to have an important impact on both the magnitude of glycaemic and insulinaemic response. Serum glucose response to fructose ingestion was not different after supplementation despite the faster emptying rate, however. This suggests that the capacity to metabolise fructose into glucose is not altered and is further supported with the observation that there were no differences in lactate concentration, suggesting that lactate production was also unaltered. Alternatively, greater uptake of glucose by cells may have occurred, though this may be unlikely as no differences were also seen for insulin secretion for either fructose or glucose ingestion despite slight variations in incretin hormone responses. The faster gastric emptying of fructose did result in a slightly higher, albeit insignificant, peak serum fructose concentration at 30 min, however. The implications of this, if any, are unknown at this stage.

Interestingly, triglyceride concentration was significantly elevated at baseline and remained elevated at pre-prandial levels at all postprandial time-points for glucose ingestion following supplementation but no difference was found between the fructose ingestion trials. Taking the glucose ingestion results alone extends the observations of Stanhope *et al*. (2011), Stanhope *et al*., (2009), Stanhope *et al*., (2008), Teff *et al*. (2009), Teff *et al*., (2004), Ngo Sock *et al*. (2010) and Bantle *et al*., (2000) in that increased fructose intake for even three days is enough to cause significant increases in fasting triglycerides. These levels were still a way from dyslipidaemia values, however. It is uncertain as to why no differences were also evident between the two fructose trials at baseline.

The accelerated emptying of fructose resulted in a trend of greater hunger suppression. It is unlikely that this was due to the hormones studied in the present study as greater ghrelin and lower GLP-1 concentrations are inconsistent with the observed hunger effects. A greater length of exposure of the intestine to fructose may have resulted in greater release of other hormones known to decrease appetite, such as PYY. In line with the lesser feelings of hunger, lower prospective food consumption was also observed with fructose ingestion following supplementation. The satiety effects of fructose ingestion was therefore greater following increased dietary intake of fructose. The absence of differences in glucose appetite measures suggests gastric emptying is an important modulatory process linked to appetite. Whether these changes in subjective feelings of appetite translate to changes in food intake need to be investigated further.

In conclusion, the results of this study show that 3 d of dietary supplementation with 120 g fructose per day results in accelerated emptying of a fructose solution but not a glucose solution which can be partly explained by moderations of gut hormone secretion. Furthermore, increased fructose ingestion for even a short period of 3 d appears to result in unfavourable changes to serum triglyceride concentration. However, the accelerated emptying rate of fructose ingestion did not result in greater appetite sensations, which may be contrary to beliefs that high fructose intake increases food ingestion. The adaptability of the gut and the effects on food intake should be further investigated.

6. THE EFFECT OF GLP-1 RECEPTOR GENETIC VARIATION ON GASTRIC EMPTYING RATE[4]

[4] Some of the data from this study contained within this chapter was presented as an oral communication and the abstract published in "Yau, A., McLaughlin, J., Maughan, R.J., Gilmore, W., Ashworth, J.J. & Evans, G.H. (2014). The influence of glucagon-like-peptide-1 receptor single nucleotide polymorphisms on gastric emptying rate in Caucasian men- a pilot study. *Proceedings of the Physiological Society*, 31, C45."

6.1. INTRODUCTION

Whilst an influence or role of genetics in obesity has been given much attention and is established to increase susceptibility of excess weight accumulation and obesity, research on the potential influence of genetics on gastric emptying rate is scarce. A recent study by Acosta and colleagues (Acosta, Camilleri, Shin, Carlson, Burton, O'Neill *et al*., 2014) has reported a common genetic variant rs17782313 in the MC4R gene to be associated with reduced gastric emptying rate and satiation. This variant had previously been found to be strongly associated with common obesity (Vogel, Boes, Reinehr, Roth, Scherag, Scherag *et al*., 2011; Loos, Lindgren, Li, Wheeler, Zhao, Prokopenko *et al*., 2008). In addition a study by Cremonini and colleagues (Cremonini, Camilleri, McKinzie, Carlson, Camilleri, Burton *et al*., 2005) has shown an association between the 779T>C polymorphism in the CCK gene and slower gastric emptying rate. On the other hand, a study by Jones, Payton, Oilier, Dockray & Thompson (2010) found no effect of common genetic polymorphisms of the CCK or CCK-1 receptor genes on gastric emptying rate following analysis of 25 participants homozygous for four different haplotype block variants identified from 520 individuals.

Given the influence of GLP-1 on gastric emptying rate and the large variation in gastric emptying rate of a glucose solution as well as the large differences in GLP-1 hormone responses to carbohydrate ingestion observed earlier in this thesis, genetic variation within a gene related to the action of GLP-1 presents a plausible area of investigation. The gastrointestinal hormone GLP-1 exerts its effects via a G-protein coupled receptor called the GLP-1R. Stimulation of the receptor by GLP-1 triggers cAMP production as the primary signal transduction pathway (Mayo, Miller, Bataille, Dalle, Goke, Thorens *et al*., 2003). The GLP-1R recognises GLP-1 specifically despite the hormone having strong sequence homology to other hormones within the glucagon-related family of peptides, and furthermore, does not bind to a number of other related peptides, including secretin (Fehmann *et al*., 1994).

Genetic polymorphisms of the GLP-1R gene have previously been investigated in relation to insulin secretion (Sathananthan, Man, Michelotto, Zinsmeister, Camilleri, Giesler *et al*., 2010) and the pathogenesis of diabetes (Beinborn, Worrall, McBride & Kopin, 2005; Tokuyama, Matsui, Egashira, Nozaki, Ishizuka & Kanatsuka, 2004; Tanizawa, Riggs, Elbein, Whelan, Doniskeller & Permutt, 1994). Only one study has previously investigated the influence of GLP-1R genetic variation on gastric emptying rate. Genetic variation in the GLP-1R gene has been shown to influence gastric emptying rate in mice (Kumar, Byerley, Volaufova, Drucker, Churchill, Li *et al*., 2008). The presence of

a nonsynonymous cysteine to tyrosine substitution at amino acid 416 of the GLP-1R gene found in CAST strain mice was associated with reduced GLP-1R expression and significantly faster gastric emptying rate by 20% compared to B6 strain mice. This was also seen for the congenic strains where gastric emptying rate was significantly higher in B6.CAST-17 congenic mice compared to homozygous B6 controls. Furthermore, administration of a GLP-1R antagonist extendin-(9-36) resulted in no increase in gastric emptying rate compared to a 10% increase in the homozygous B6 control mice. The GLP-1R gene is therefore a plausible candidate gene for a genetic association study on gastric emptying rate in humans. The human GLP-1R gene consists of 13 exons interrupted by 12 introns (Wilmen, Walkenbach, Fuller, Lankat-Buttgereit, Goke & Goke, 1998) and is situated on chromosome 6, band p21.1 (Stoffel, Espinosa, Lebeau & Bell, 1993). Figure 46 depicts the genomic organisation of the GLP-1R gene. A major transcription start point and a minor transcription start point 42 base pair (bp) and 360 bp upstream of the translation initiation site, respectively has been reported (Lankat-Buttgereit & Goke, 1997). Three putative Sp1 binding sites have also been located in the proximal 5' flanking sequence at -108, -173 and -389 bp from the translation initiation codon (Lankat-Buttgereit & Goke, 1997). The receptor is 463 amino acids in length and is highly conserved between species, with 90% being identical to rat GLP-1R (Dillon, Tanizawa, Wheeler, Leng, Ligon, Rabin *et al*., 1993) and approximately 95% homology with mice GLP-1R.

The primary aim of this study was to investigate the influence of genetic variation in the GLP-1R gene on gastric emptying rate of a glucose solution in humans. Secondary aims of this study were to determine whether BMI or body fat percentage may be influenced by GLP-1R genetic variation and whether these variables are associated with gastric emptying rate.

6.2. METHODS

6.2.1. Participants

Fifty healthy UK Caucasian male volunteers aged between 18-35 y (mean ± S.D, age 23 ± 5 y, height 178.1 ± 6.9 cm, BM 75.49 ± 11.16 kg, BMI 23.78 ± 3.25 $kg.m^{-2}$, and estimated body fat percentage 18.9 ± 6.3%) participated in the present investigation.

6.2.2. Experimental trial

Participants reported to the laboratory for one experimental trial. In addition to the pre-trial conditions outlined in general methods, participants were also asked to record their food

and drink intake as well as physical activity during the 24 h prior to their experimental visit.

Upon arrival at the laboratory, participants were asked to completely empty their bladder into a container from which a 5 mL urine sample was retained for later analysis of osmolality. Body mass was subsequently recorded. Following this a single 4 mL blood sample was obtained by venepuncture of an antecubital vein into an EDTA vacutainer (Beckton Dickinson, UK). Once this procedure was completed, participants were seated and then ingested 595 mL of a 6% glucose solution consisting of 39.6 g glucose monohydrate (MyProtein.com) dissolved in commercially available natural mineral water (Evian, Danone Ltd, France). Participants were given a maximum of 2 min to consume the test solution and instructed to consume it as quickly as they were able to. The drink solution was prepared fresh in the morning prior to the trial and given at room temperature. A 5 mL sample of the drink was retained for later analysis of osmolality. Participants remained seated throughout the 60 min post ingestion sampling period where gastric emptying rate and appetite ratings of hunger, fullness and prospective food consumption were assessed as described in general methods. Following all sample collections at 60 min, participants were asked again to completely empty their bladder into a container and a 5 mL sample was retained for later analysis.

6.2.3. Genotyping

Genomic DNA was extracted from 3 mL of whole blood before a second set of genomic DNA was extracted from an additional 300 µl of blood. Both extractions were performed using Flexigene DNA Kit (Qiagen, West Sussex, UK) according to the manufacturer's instructions except for the final step of resuspension in 300 µl and 50 µl of water, for the two extractions respectively. The second extraction was performed as a precaution due to the first set of samples having initially been resuspended and normalised in rehydration buffer as according to the manufacturer's protocol instead of water as required. In an attempt to rectify this, the normalised samples were left uncapped in a cupboard at room temperature for a number of weeks and those with very high volumes in a water bath at 50ºC for half a day in an attempt to evaporate as much of the fluid as possible. The samples were subsequently re-precipitated and re-purified by repetition of the appropriate steps in the protocol and resuspended in water. Due to approximately 50% of the samples not appearing to have re-precipitated sufficiently and as a result of the above conditions the samples were subjected to, the quality and extent of DNA fragmentation was unknown. Both sets of extracted DNA were quanitified using a NanoDrop 2000 (Thermo Scientific,

Loughborough, UK) and normalised to a concentration of 40 ng/µl and stored at -20ºC before working concentrations of 20 ng/µl were prepared in 96 well plate format prior to use.

Twenty-eight tag SNPs in the GLP-1R locus incorporating 10,000 bp upstream and downstream of the major transcription initiation site and the last exon, respectively, (Chr6: 39114595. . 39173498) were selected from HapMap (www.hapmap.org -HapMap Data release 27 / phase II+III, Feb09, on NCBI B36 assembly, dbSNP b126). The Tagger algorithm for multi-marker tagging with $r^2 > 0.8$ and minor allele frequency > 0.1 was used (de Bakker, Yelensky, Pe'er, Gabriel, Daly & Altshuler, 2005). Furthermore, three additional nonsynonymous SNPs were selected based on previous literature. One of two SNPs found to be associated with insulin secretion in response to exogenous infusion of GLP-1 by Sathananthan *et al*. (2010) and not already in the generated list of 28 tag SNPs was genotyped. The other two additional SNPs genotyped were two in very close proximity (two amino acids upstream and three amino acids downstream) to the equivalent locus of the SNP found in mice to be associated with gastric emptying rate (Kumar *et al*., 2008). All 31 SNPs (Table 5 and Figure 46) were genotyped using Sequenom MassARRAY iPLEX GOLD analysis. Forward and reverse primers as well as extension probes were designed using Sequenom Assay Design Suite (v1.0) which produced two appropriate assay plexes; plex one containing 24 SNPs and plex two containing 7 SNPs (Table 5). Primers were purchased from Metabion International AG (Martinsreid, Germany).

Briefly, an initial locus-specific amplification was performed using polymerase chain reaction (PCR; GeneAmp PCR System 9700, Applied Biosystems) carried out in a total volume of 5 µl containing 40 ng of DNA and final concentrations of 1.25x buffer, 1.0 mM $MgCl_2$, 500 µM dNTP mix, 100 nM of each forward and reverse primers, 0.1 U/µL Hotstart *Taq*. PCR conditions consisted of an initial denaturing step at 94°C for 5 min, followed by 40 cycles of denaturing at 94°C for 20 sec, annealing at 56°C for 30 sec and extension at 72°C for 1 min and then a final extension step at 72°C for 3 min. Unincorporated dNTPs within the PCR products were then dephosphorylated by treatment with Shrimp Alkaline Phosphatase (SAP) through the addition of 2 µl SAP mixture composed of 0.17 µl 10x hME buffer, 0.3 µl SAP enzyme 1.7 U/µl and water, and incubated for 40 min at 37°C and 5 min at 85°C. A single base extension step was then performed with the addition of 2 µl reaction mix containing a concentration of 50 µM of each ddNTP, 3.3 µM:6.6 µM extension probe from low:high mass (2.8 µM:5.6 µM for plex 2), 1.0x buffer and 1.25 U Thermo Sequenase enzyme. Probe extension conditions consisted of a two-step 200 short cycle program involving initial denaturing at 94°C for 30

sec, 40 cycles of denaturing at 94°C for 5 sec, annealing at 52°C for 5 sec and extension at 80°C for 5 sec where the annealing and extension steps also cycled 5 times, before a final extension at 72°C for 3 min. Resulting products were diluted with 20 µl water and desalted with 6 mg resin by gentle inversion for 10 min before centrifugation at 4000 rpm for 5 min. Products were subsequently nano-dispensed (Samsung Sequenom MassARRAY Nanodispenser) onto a 384-element SpectroCHIP II bioarray and analysed by MALDI-TOF mass spectrometry.

6.2.4. Data analysis

Genotype results were visually checked and where required, when no automated genotype calling successfully took place, appropriate manual genotype assignment was made upon spectrum inspection. Where genotype results were available for both sets of extraction samples, concordance between the two extractions enabled further verification of results.

Differences in gastric emptying $T_{½}$ and T_{lag}, BMI and body fat percentage were examined by genotype and phenotype. Normality tests indicated that the majority of data were not normally distributed. Furthermore, as group sizes were unequal, non-parametric statistical analysis comprising of Kruskal-Wallace and Mann-Whitney U tests were utilised, respectively. Where appropriate, post-hoc tests of Mann-Whitney U with Bonferroni correction were applied. Pearson's product moment correlations were used to investigate relationships between gastric emptying rate and BMI and body fat percentage. All data was analysed using SPSS Statistics for Windows version 19 (IBM, New York, US). Statistical significance was accepted at the 5% level and results presented as median and quartiles unless stated otherwise.

Table 5. List of SNPs analysed and primers used. Unshaded SNPs and primers in plex 1. Shaded primers in plex 2.

	SNP ID	Pos. from major transcription site (bp)	Forward primer	Reverse primer	Extension probe
1	rs7738586	-9,033	ACGTTGGATGACACCCAGACTGACGTTATC	ACGTTGGATGTTGTGCAAAAGCAGCCCAAG	TCAAAGTGATTGTCACCATAAG
2	rs9380825	-5,610	ACGTTGGATGTGAGCCAGGAAGTGATGTTC	ACGTTGGATGTCTCATCCAGGCAGCAAGTA	cccCTGCACCAGAGCCCCT
3	rs9296274	-1,455	ACGTTGGATGTTTGGATATCGTGGCTGGAG	ACGTTGGATGTGCCTGGGTCTTCTAGCTTC	gggtGTGGAAATCTTGGACCA
4	rs926674	2,930	ACGTTGGATGACTCACACATACCTGGGAAC	ACGTTGGATGATACAGACAGGTAGTCTGAG	tagcAGGGAGTAGGCTATATGA
5	rs2268657	3,969	ACGTTGGATGGTTCTGCCGTCCATAAAATG	ACGTTGGATGTACAGGGCTTGAGAAGTCAC	ggaagTGGGCATATCATTCTTCTCA
6	rs13202369	6,125	ACGTTGGATGTGATCCACCAGGACTTGCTC	ACGTTGGATGTAACAGCTGCAAAGGTGTTG	gacaTCCTCAGCTGTGGCTAAT
7	rs3799707	6,937	ACGTTGGATGTTTGGTTGCTGTGTCAGAGG	ACGTTGGATGGCACTCACTTACAGATGCAC	AGATGCACTCAACACA(Inosine)C
8	rs10305432	7,057	ACGTTGGATGTCTTTGTAGCCCTGAACGCC	ACGTTGGATGAGTCCTTCAGATCAGTGACC	CCGCACACCTTGCGA
9	rs9283907	10,130	ACGTTGGATGGCTCCTATCATCACACCTTG	ACGTTGGATGCCAGAGCATAACCTCATGCC	aagaA(Inosine)CAACTGGCCCAGAA
10	rs742764	13,261	ACGTTGGATGTCTCAGCTTCTGCATCTGTA	ACGTTGGATGTGTGGAGAGCTGCTCATGAA	ggagcGCTGCTCATGAATCCATTA
11	rs2254336	16,262	ACGTTGGATGCTGGTCTAAAAGGAGTACAC	ACGTTGGATGAAGGTAGGAGCTGGGTATTC	ggAGGCTGCATACGACCA
12	rs910163	16,847	ACGTTGGATGTGAGCCTCAGCCCAGAATAG	ACGTTGGATGCAGGGATAGCCCTCAGAATG	tATGGGGAGGAAGGGG
13	rs3765467*	17,022	ACGTTGGATGAACCCCGCCTCAACTCACTC	ACGTTGGATGTGCAGAAGGACAACTCCAGC	AACTCACTCT(Inosine)TCCCCT
14	rs6923761	17,499	ACGTTGGATGTTCTCTGCTCTGGTTATCGC	ACGTTGGATGGAGTTAGGATGAAGCAGCCC	GGGCCACCTTACCTGAAGC
15	rs7766663	19,209	ACGTTGGATGAGCAATAGGTCTGCATGTGG	ACGTTGGATGCTGGTCTCCAATCTCTGGC	CCTAGCTAATTGAGAGGC
16	rs932443	25,761	ACGTTGGATGGTGAATGAAGGAGTGGCAAG	ACGTTGGATGTCCTCCCTAATCTGCCATTG	ggattGTGTGGAACAGGAAAACTC
17	rs2268646	26,943	ACGTTGGATGCCAACTGTGTCAGAGTCCTA	ACGTTGGATGGTGATGCCAGGAGGCCTTG	cccaTTCCACTTGCACATGAA
18	rs2300614	32,400	ACGTTGGATGTCCAAACCTAGGGCAGGTTC	ACGTTGGATGCCCTGCTAAAATTCTTATTTC	TCTTTCTGATCTTCAGTGTT
19	rs2268641	33,693	ACGTTGGATGCTGGGTCCTCTAAGACCTGT	ACGTTGGATGCAAAGAGTGGCCCATAAATG	ggggAAGACCTGTCCCAGGA
20	rs2268640	33,811	ACGTTGGATGTGCACTTCCTCGTTTGCATC	ACGTTGGATGTCCTCCTCCACTGCCATATC	CACTGCCATATCCTCAAAATGA
21	rs2268639	34,049	ACGTTGGATGGATAGAGAAGTGAGAAACGG	ACGTTGGATGATGAGGAGCAGAGGCCTGTA	ggcaCTGCTGCCACCTTGTCATCT
22	rs2206942	34,866	ACGTTGGATGAATTGGGAAGCTCATTCACC	ACGTTGGATGGGCAAGTCATTTTGCCTCCC	ggcGCTCATTCACCTTCATTTAC
23	rs2894420	36,523	ACGTTGGATGAACAGGGATCCTGGCTGAC	ACGTTGGATGAGTGAGGGCTTCTCAACTG	CACTGCAGTGTCTCTCT
24	rs199796313†	37,124	ACGTTGGATGTCCTTTTCCCATGGAAGGTC	ACGTTGGATGTGGATGTGCAAGTGCTCAAG	GGTCCAGCTGGAATTT
25	rs200691429†	37,140	ACGTTGGATGAAGGTCCAGCTGGAATTTCG	ACGTTGGATGTGGATGTGCAAGTGCTCAAG	gGGAAGAGCTGGGAGC
26	rs4714211	39,117	ACGTTGGATGAAGGCACCCCTTATTTGCTG	ACGTTGGATGACTTGCACCAGCACTGTTTC	ccccTTTGCTGTCTCTTCGT
27	rs10305525	39,577	ACGTTGGATGAATGGCACTGCACTCTTTCC	ACGTTGGATGCATTGCATTCAATAGTTCCC	aATTCAATAGTTCCCAGACCT
28	rs9296291	40,356	ACGTTGGATGGATGGTGAAAGTGTCATCTC	ACGTTGGATGAAGACAAGGATGAATGAAG	gTGAATGAAGTACCAGTGT
29	rs9968886	43,905	ACGTTGGATGACAGTGAGGTTTTCCCCATC	ACGTTGGATGTCATGTAGTCCAGCTTGTGC	GCTTGTGCTGCTAGTT
30	rs2143733	44,923	ACGTTGGATGCATCTAATCGATGGGTAGC	ACGTTGGATGCAGAACCCTTCTGAACCTTC	GAAATTGAATTTACAGCTTTAATAAA
31	rs9296292	46,417	ACGTTGGATGTCACAATATGTTTGGCACTG	ACGTTGGATGGCTTTGTTTTGCAGAGCTTG	TGGCACTGCCAAACT

*Additional missense SNP associated with insulin secretion in response to exogenous infusion of GLP-1 by Sathananthan *et al*. (2010)

†Additional missense SNPs in close proximity to equivalent SNP associated with gastric emptying rate in mice (Kumar *et al*., 2008).

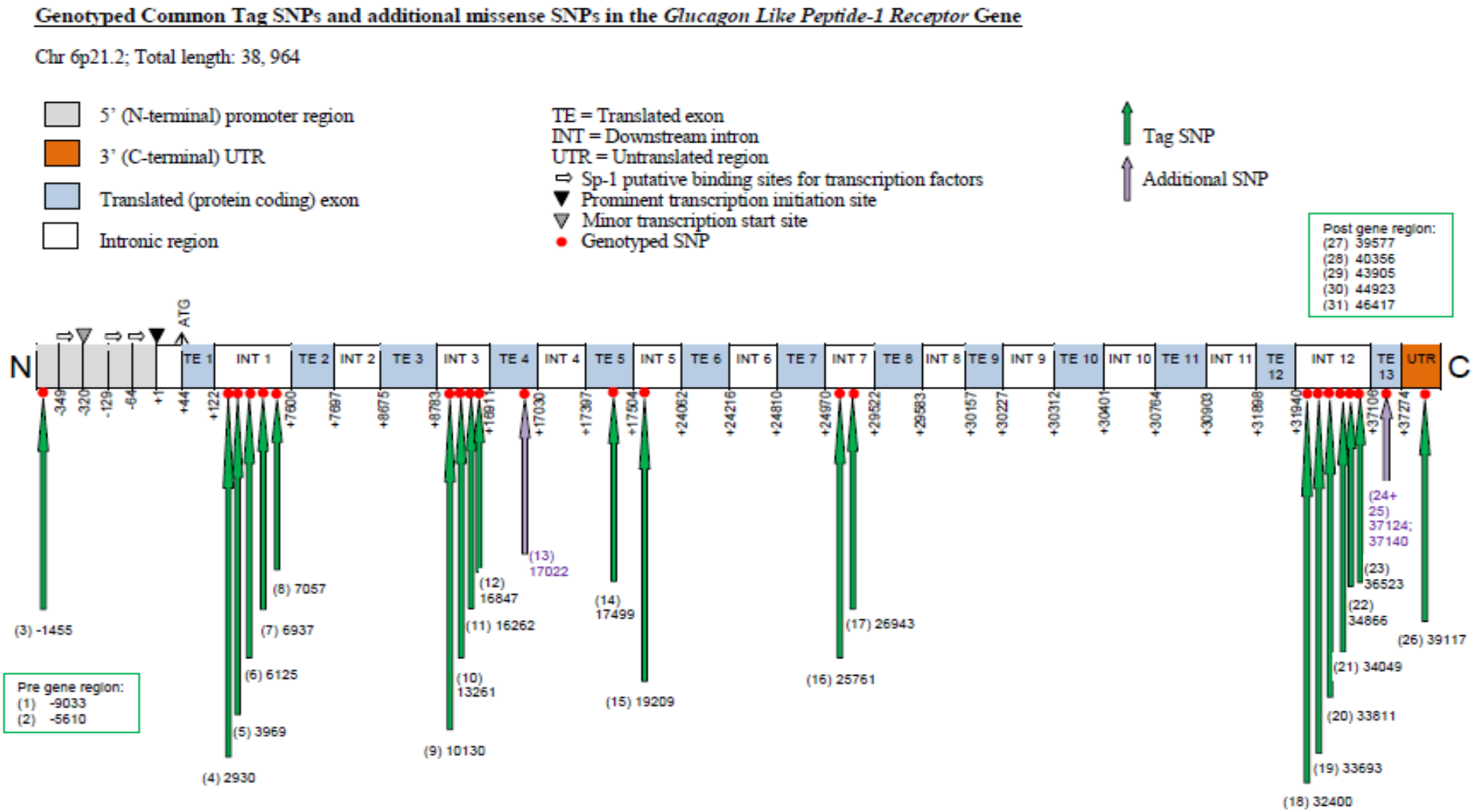

Figure 46. Schematic representation of the genomic organisation of the GLP-1R gene and SNPs genotyped

6.3. RESULTS

6.3.1. Participant exclusion

One participant was excluded from analysis due to an abnormally high T½ gastric emptying result affected by what appeared to be an inadequate end-expiratory sample at 30 min. A second participant was excluded from further analysis due to multiple genotype failures. Forty-eight participants were therefore included in the analysis (mean ± SD age 23 ± 5 y, height 178.2 ± 6.9 cm, BM 75.82 ± 11.24 kg, BMI 23.9 ± 3.3 $kg.m^{-2}$, estimated body fat percentage 19.0 ± 6.2%).

6.3.2. Hydration status and drink osmolality

The majority of participants were well hydrated according to their pre-trial urine osmolality. Five participants were classified as hypohydrated indicated by a urine osmolality > 900 mOsmol/kg. However, these participants were not excluded from analysis due to the absent effect of hypohydration on gastric emptying rate (Ryan, Lambert, Shi, Chang, Summers & Gisolfi, 1998). Mean ± SD pre-trial urine osmolality was 489 ± 280 mOsmol/kg. Mean ± SD drink osmolality was 371 ± 3 mOsmol/kg.

6.3.3. SNP genotyping

Twenty-seven out of the 31 SNPs were successfully analysed for variants. No variants occurred amongst the participants for three SNPs, Tag SNP 3 within the promoter region (rs9296274) and SNPs 24 (rs199796313) and 25 (rs200691429), two of the three additional missense SNPs selected. Genotyping failed in all participants for SNP 13 (rs3765467), one of the three additional missense SNPs. The occasional failure to successfully genotype one participant occurred in five SNPs, reducing the total participant number (*n)* to 47 instead of 48. Frequencies of each genotype are shown in Table 6. All SNPs except SNP 4 (rs926674; $P = 0.012$) were in Hardy-Weinberg equilibrium.

Table 6. SNP genotype frequencies

	SNP ID	Genotype Frequencies						
		Homozygous minor allele		Heterozygous		Homozygous major allele		
		Genotype	*n*	Genotype	*n*	Genotype	*n*	Total
1	rs7738586	AA	0	CA	10	CC	38	48
2	rs9380825	AA	5	AG	28	GG	15	48
3	rs9296274	--	--	--	--	GG	48	48
4	rs926674	TT	3	TC	6	CC	39	48
5	rs2268657	GG	9	AG	27	AA	11	47
6	rs13202369	GG	2	AG	21	AA	25	48
7	rs3799707	TT	3	GT	19	GG	26	48
8	rs10305432	CC	3	CT	19	TT	26	48
9	rs9283907	AA	1	AG	9	GG	38	48
10	rs742764	CC	8	TC	25	TT	14	47
11	rs2254336	TT	9	TA	23	AA	16	48
12	rs910163	CC	3	TC	18	TT	27	48
13	rs3765467*							
14	rs6923761	AA	7	GA	28	GG	13	48
15	rs7766663	GG	10	GT	23	TT	15	48
16	rs932443	GG	5	AG	21	AA	22	48
17	rs2268646	AA	0	AG	10	GG	38	48
18	rs2300614	TT	5	CT	21	CC	22	48
19	rs2268641	AA	4	AG	21	GG	22	47
20	rs2268640	CC	3	TC	18	TT	27	48
21	rs2268639	TT	3	TA	22	AA	22	48
22	rs2206942	AA	6	AG	24	GG	18	48
23	rs2894420	AA	9	AG	27	GG	11	47
24	rs199796313†	--	--	--	--	CC	48	48
25	rs200691429†	--	--	--	--	GG	48	48
26	rs4714211	GG	7	AG	27	AA	13	47
27	rs10305525	AA	0	CA	9	CC	39	48
28	rs9296291	CC	3	TC	17	TT	28	48
29	rs9968886	AA	0	GA	12	GG	36	48
30	rs2143733	GG	7	GT	27	TT	14	48
31	rs9296292	CC	4	CT	20	TT	24	48

*Additional missense SNP associated with insulin secretion in response to exogenous infusion of GLP-1 by Sathananthan *et al*. (2010). †Additional missense SNPs in close proximity to equivalent SNP associated with gastric emptying rate in mice (Kumar *et al*., 2008).
Pink highlighted SNPs, no variants present. Red highlighted SNP failed to genotype.

6.3.4. Gastric emptying

Mean ± SD gastric emptying rate for all participants was 68 ± 16 min and 41 ± 8 min for $T_{½}$ and T_{lag}, respectively. Median (quartiles) gastric emptying rate for all participants was 63 (55-78) min and 40 (36-44) min for $T_{½}$ and T_{lag}, respectively.

6.3.4.1. By genotype

Results for T_{lag} according to genotype are shown in Table 7. Results for $T_{½}$ according to genotype are shown in Table 8. Non-parametric statistical analysis revealed significant differences in median gastric emptying T_{lag} for SNP 10 rs742764 (Figure 55) and SNP 11 rs2254336 (Figure 56). For SNP 10 rs742764, gastric emptying T_{lag} was significantly faster in genotype CC compared to genotype TT (P = 0.006) and TC (P = 0.006) by 15%. Half emptying time was also close to significance with differences of 18% (P = 0.061). For SNP 11 rs2254336, gastric emptying T_{lag} was significantly slower in genotype AA compared to

genotype TT (P = 0.04) and TA (P = 0.036) by 19% and 10%, respectively. Half emptying time showed a slight trend to be slower than both other groups by 20% and 15% but this did not reach statistical significance (P = 0.138). No significant differences in gastric emptying rate were seen between genotypes in all other SNPs (Figure 47 to Figure 54 and Figure 57 to Figure 73) although $T_{½}$ tended toward significance for SNP 9 rs9283907 (P = 0.054) and T_{lag} tended to significance for SNP 5 rs2268657 (P = 0.087) and SNP 15 rs7766663 (P = 0.076).

Table 7. Gastric emptying T_{lag} results according to genotype. Values are minutes.

		Homozygous Minor Allele					Heterozygous					Homozygous Major Allele					
	SNP ID	**Genotype**	**Mean**	**SD**	**Median**	**Quartiles**	**Genotype**	**Mean**	**SD**	**Median**	**Quartiles**	**Genotype**	**Mean**	**SD**	**Median**	**Quartiles**	***P*-value**
1	rs7738586	---	--	--	--	--	CA	40	9	37	34-41	CC	42	8	40	37-45	0.274
2	rs9380825	AA	43	8	42	61-80	AG	42	9	41	37-45	GG	39	7	37	36-40	0.349
4	rs926674	TT	39	5	37	37-41	TC	38	3	38	36-40	CC	42	9	41	36-46	0.448
5	rs2268657	GG	43	9	42	37-44	AG	42	9	38	37-46	AA	36	5	37	34-39	0.087
6	rs13202369	GG	34	8	34	31-36	AG	42	9	41	37-49	AA	41	8	39	36-42	0.295
7	rs3799707	TT	38	8	42	36-43	GT	40	9	40	35-43	GG	42	8	40	37-45	0.696
8	rs10305432	CC	36	2	36	35-37	CT	42	9	41	39-43	TT	41	9	40	35-46	0.267
9	rs9283907	AA	29	--	29	29-29	AG	39	8	37	35-41	GG	42	8	41	37-44	0.158
10	rs742764	CC	34	4	35*	30-36	TC	43	9	41	39-45	TT	42	7	41	37-46	**0.008**
11	rs2254336	TT	38	11	36	34-41	TA	40	8	39	35-42	AA	45	7	43†	39-49	**0.031**
12	rs910163	CC	38	3	37	37-39	TC	42	8	40	36-45	TT	41	9	41	36-44	0.788
14	rs6923761	AA	44	8	43	41-48	GA	41	8	40	37-43	GG	40	10	37	34-41	0.222
15	rs7766663	GG	37	8	36	31-40	GT	42	8	40	38-44	TT	43	8	42	37-48	0.076
16	rs932443	GG	38	3	37	36-41	AG	42	10	40	38-45	AA	41	8	41	35-44	0.634
17	rs2268646	--	--	--	--	--	AG	43	8	40	37-48	GG	41	8	40	35-43	0.493
18	rs2300614	TT	36	4	36	35-37	CT	41	8	40	37-44	CC	42	9	42	36-46	0.210
19	rs2268641	AA	38	2	37	37-38	AG	41	8	40	35-44	GG	41	9	41	35-44	0.724
20	rs2268640	CC	38	3	37	37-39	TC	41	9	40	34-45	TT	41	8	41	37-44	0.741
21	rs2268639	TT	38	3	37	37-39	TA	41	8	40	35-44	AA	41	8	41	36-44	0.816
22	rs2206942	AA	44	9	39	37-51	AG	41	7	40	36-43	GG	41	10	41	32-46	0.878
23	rs2894420	AA	44	11	43	39-49	AG	39	6	39	36-42	GG	42	9	37	36-48	0.470
26	rs4714211	GG	37	9	36	33-39	AG	42	8	40	37-45	AA	43	9	41	37-44	0.244
27	rs10305525	--	--	--	--	--	CA	39	7	37	36-41	CC	42	9	40	36-45	0.369
28	rs9296291	CC	38	3	37	37-39	TC	42	9	40	35-45	TT	41	9	41	37-43	0.812
29	rs9968886	--	--	--	--	--	GA	41	9	37	36-45	GG	41	8	41	36-43	0.543
30	rs2143733	GG	42	11	37	37-48	GT	40	7	39	35-43	TT	43	9	42	39-48	0.549
31	rs9296292	CC	36	5	37	34-38	CT	41	9	39	34-44	TT	43	8	41	38-45	0.166

Reported *P*-values are for median data. *Significantly faster than other two genotypes ($P < 0.01$). †Significantly slower than other two genotypes ($P < 0.05$).

Table 8. Gastric emptying $T_{½}$ results according to genotype. Values are minutes.

		Homozygous Minor Allele					Heterozygous					Homozygous Major Allele					
	SNP ID	**Genotype**	**Mean**	**SD**	**Median**	**Quartiles**	**Genotype**	**Mean**	**SD**	**Median**	**Quartiles**	**Genotype**	**Mean**	**SD**	**Median**	**Quartiles**	***P*-value**
1	rs7738586	---	--	--	--	--	CA	66	15	62	56-72	CC	68	17	64	55-79	0.919
2	rs9380825	AA	70	13	71	61-80	AG	69	18	64	56-80	GG	65	15	60	55-70	0.650
4	rs926674	TT	59	6	59	56-62	TC	64	12	60	55-71	CC	69	17	64	56-82	0.569
5	rs2268657	GG	73	14	72	59-83	AG	68	17	61	56-73	AA	62	14	59	53-67	0.203
6	rs13202369	GG	70	32	70	58-81	AG	68	17	62	54-77	AA	68	16	64	56-73	0.935
7	rs3799707	TT	62	9	61	57-66	GT	67	20	59	54-83	GG	69	14	66	59-78	0.452
8	rs10305432	CC	65	16	59	56-71	CT	68	17	65	57-75	TT	68	16	62	55-80	0.852
9	rs9283907	AA	41	--	41	41-41	AG	62	18	56	54-62	GG	70	15	67	59-82	0.054
10	rs742764	CC	57	12	54	53-59	TC	71	18	66	56-80	TT	70	14	66	59-81	0.061
11	rs2254336	TT	63	17	59	53-68	TA	66	16	62	54-75	AA	73	16	71	62-85	0.138
12	rs910163	CC	57	4	59	56-60	TC	71	19	66	54-82	TT	67	15	62	57-73	0.431
14	rs6923761	AA	74	12	72	66-81	GA	66	15	62	56-74	GG	68	22	59	54-83	0.297
15	rs7766663	GG	63	19	57	53-66	GT	67	17	62	54-79	TT	72	14	71	61-81	0.168
16	rs932443	GG	65	12	60	59-68	AG	71	19	65	54-83	AA	66	15	62	57-72	0.820
17	rs2268646	--	--	--	--	--	AG	71	17	67	57-87	GG	67	16	63	54-76	0.485
18	rs2300614	TT	56	3	54	53-59	CT	70	19	65	54-83	CC	69	15	66	58-73	0.120
19	rs2268641	AA	57	4	57	54-59	AG	69	17	65	54-80	GG	68	16	63	58-73	0.283
20	rs2268640	CC	57	4	59	56-60	TC	70	19	66	54-82	TT	68	15	62	57-73	0.453
21	rs2268639	TT	57	4	59	56-60	TA	68	18	65	55-79	AA	68	15	63	56-73	0.475
22	rs2206942	AA	72	23	60	57-86	AG	68	15	65	55-78	GG	67	17	63	55-73	0.959
23	rs2894420	AA	76	16	72	64-92	AG	64	12	62	55-72	GG	69	22	59	54-81	0.187
26	rs4714211	GG	61	18	54	54-60	AG	68	16	64	56-75	AA	71	16	68	59-89	0.233
27	rs10305525	--	--	--	--	--	CA	64	18	59	55-68	CC	69	16	64	55-79	0.412
28	rs9296291	CC	57	4	59	56-60	TC	71	19	66	54-83	TT	67	16	62	56-72	0.386
29	rs9968886	--	--	--	--	--	GA	69	19	61	56-92	GG	68	16	65	54-74	0.934
30	rs2143733	GG	66	20	59	55-77	GT	68	17	64	55-79	TT	69	15	65	59-73	0.791
31	rs9296292	CC	55	6	56	52-59	CT	68	19	65	54-81	TT	70	15	66	60-77	0.125

Reported *P*-values are for median data.

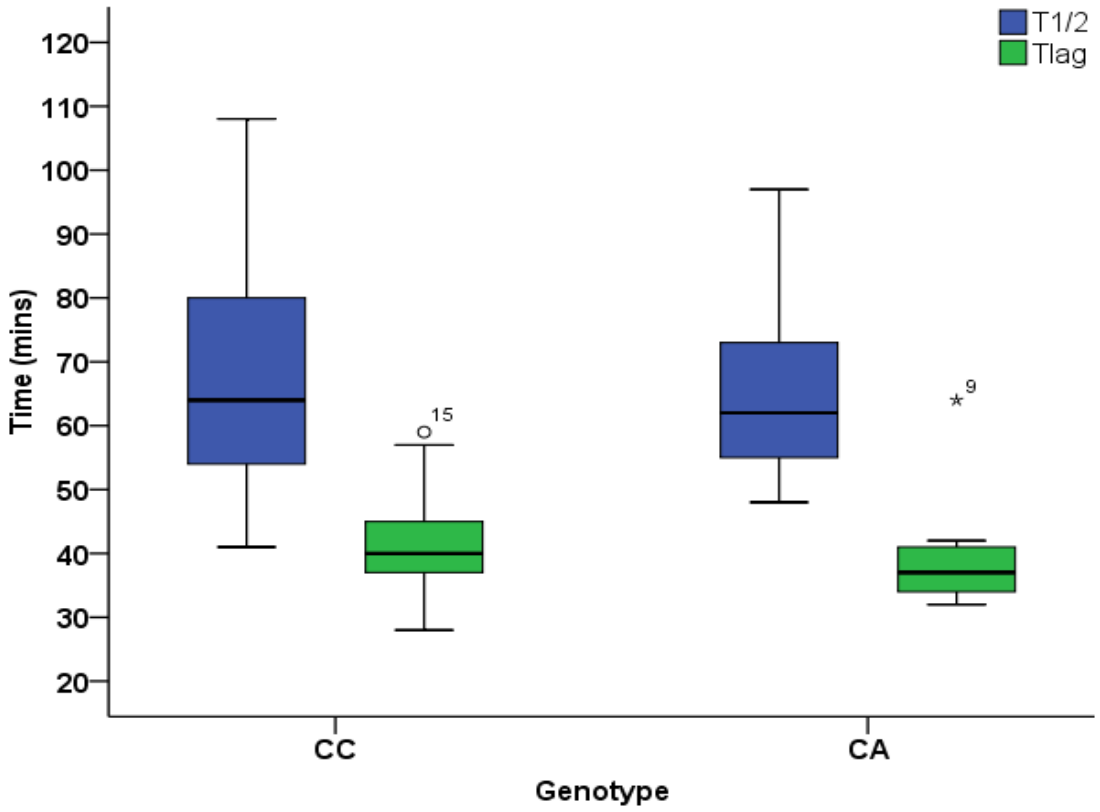

Figure 47. Gastric emptying rate boxplot for SNP 1 rs7738586 according to genotype

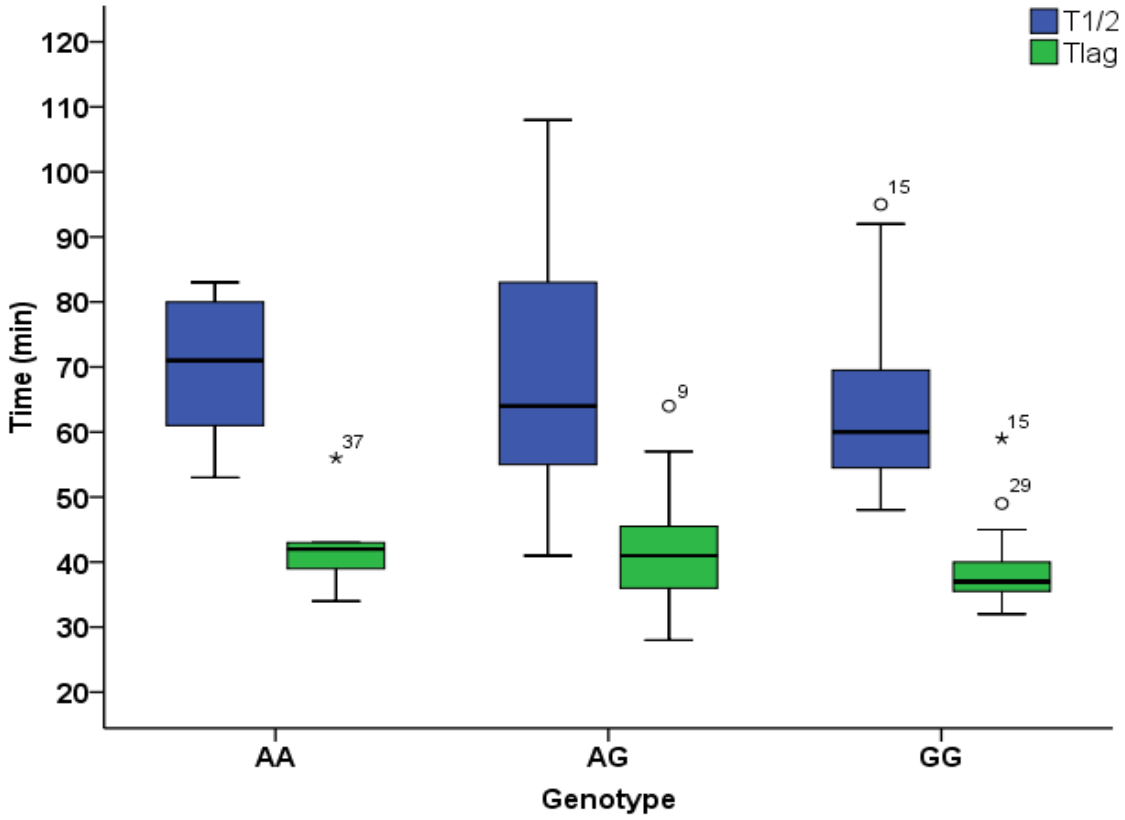

Figure 48. Gastric emptying rate boxplot for SNP 2 rs9380825 according to genotype

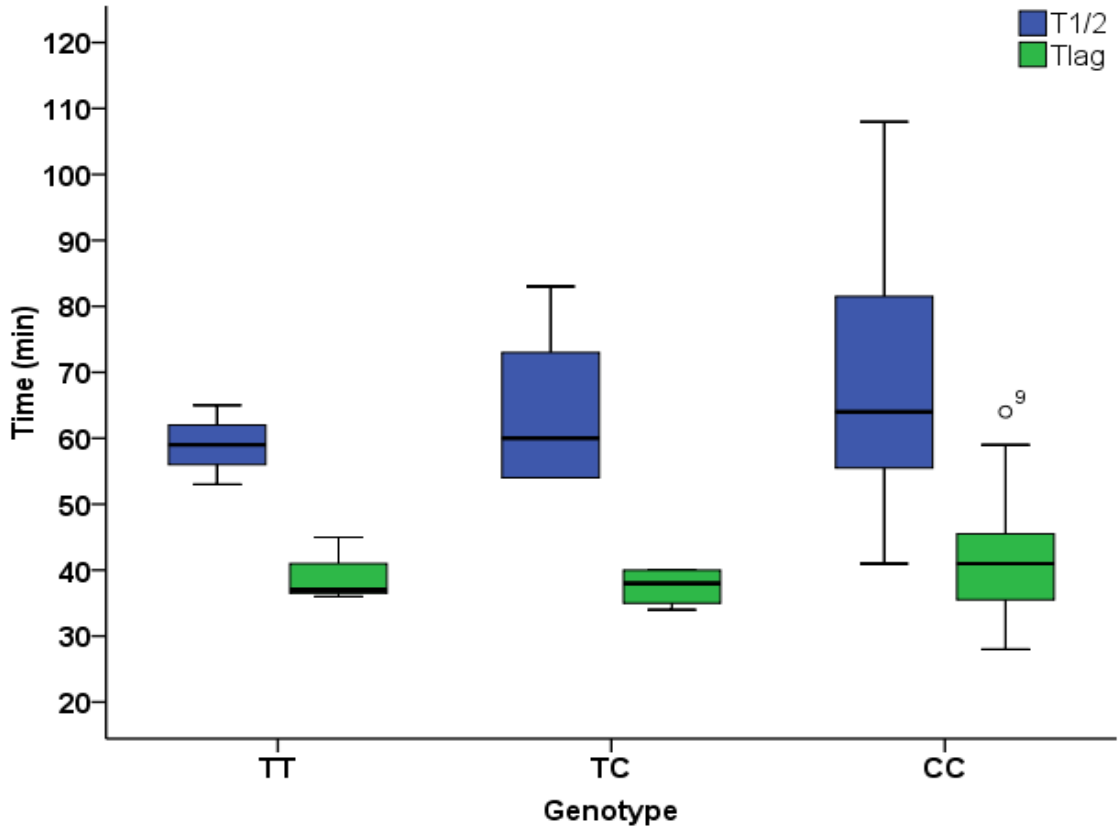

Figure 49. Gastric emptying rate boxplot for SNP 4 rs926674 according to genotype

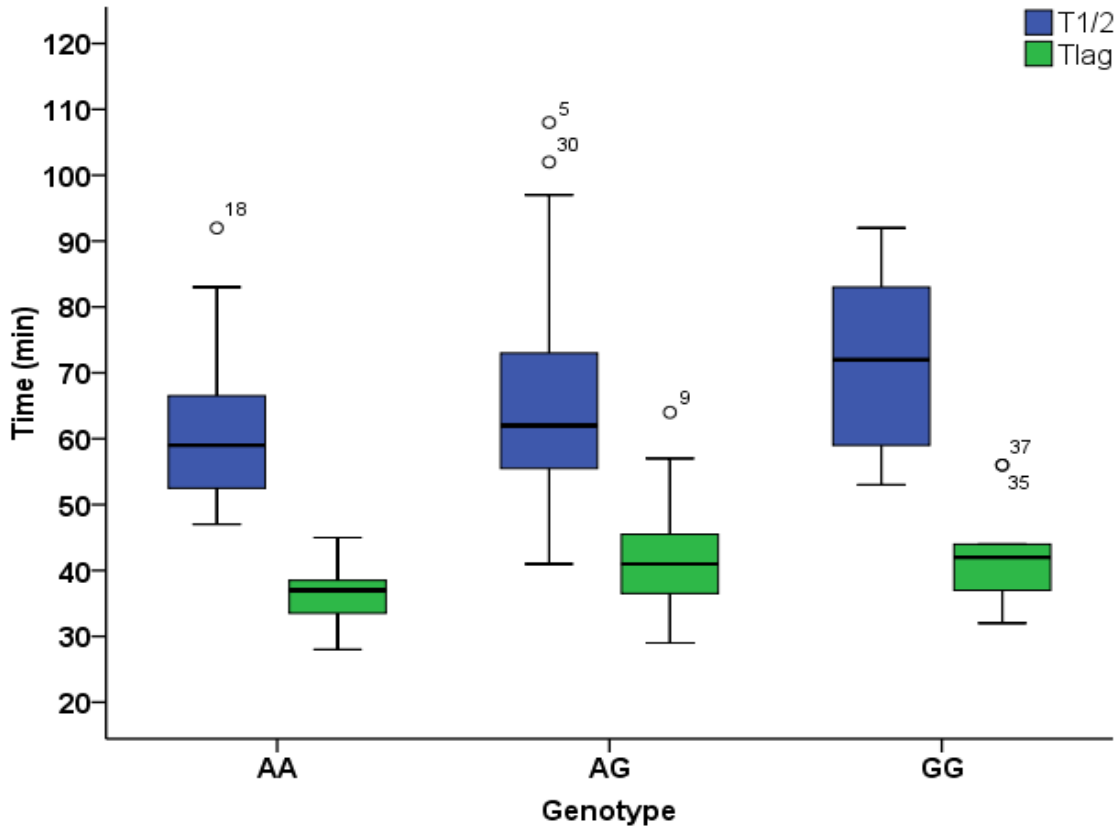

Figure 50. Gastric emptying rate boxplot for SNP 5 rs2268657 according to genotype

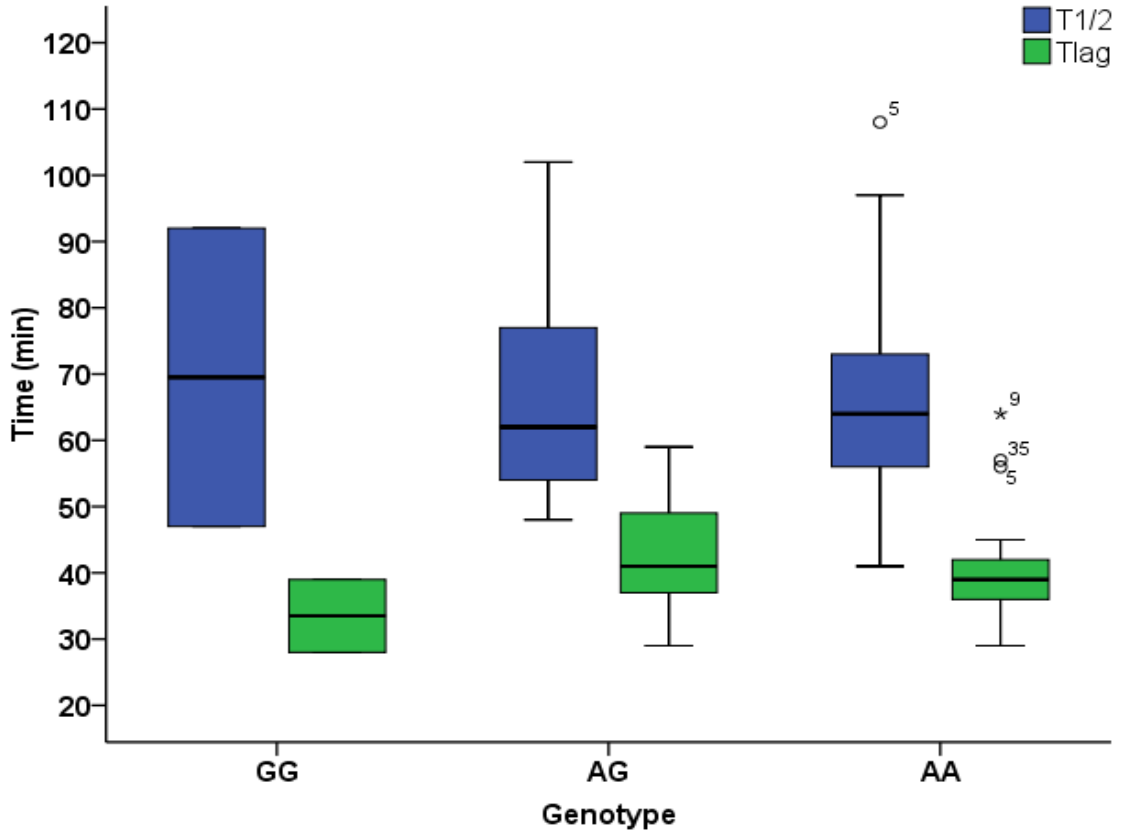

Figure 51. Gastric emptying rate boxplot for SNP 6 rs13202369 according to genotype

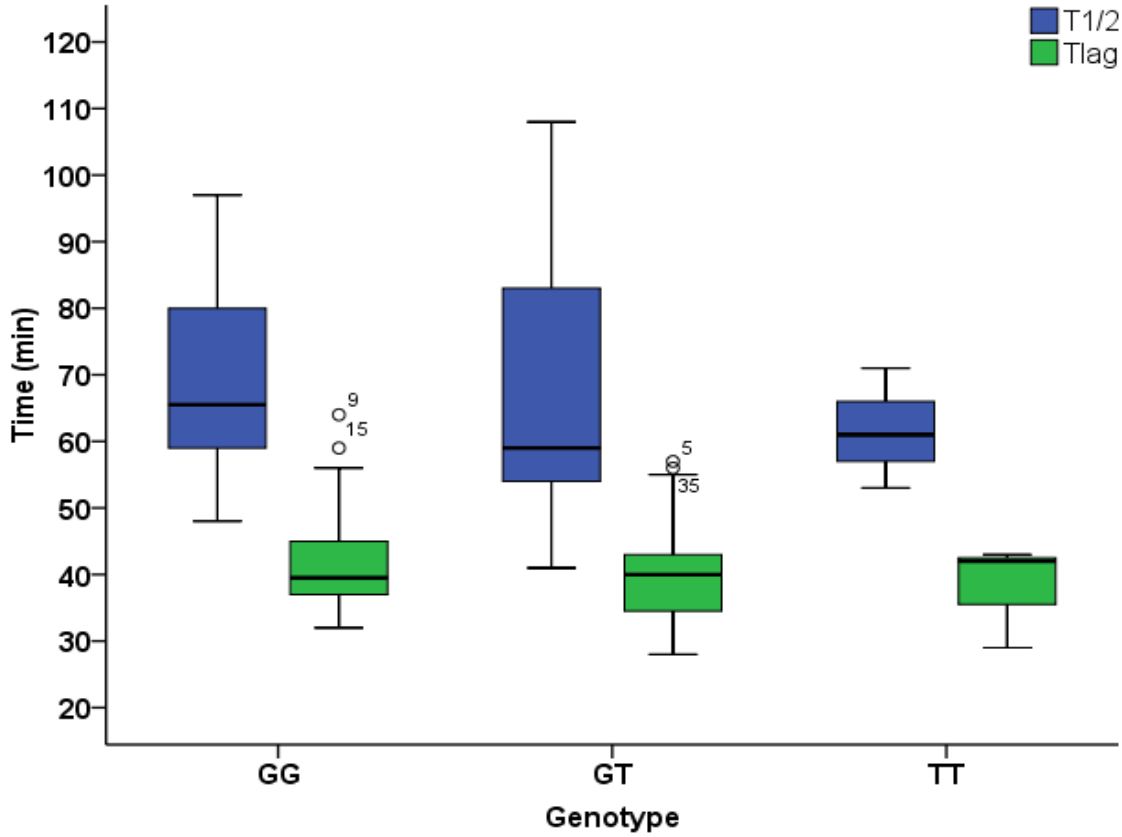

Figure 52. Gastric emptying rate boxplot for SNP 7 rs3799707 according to genotype

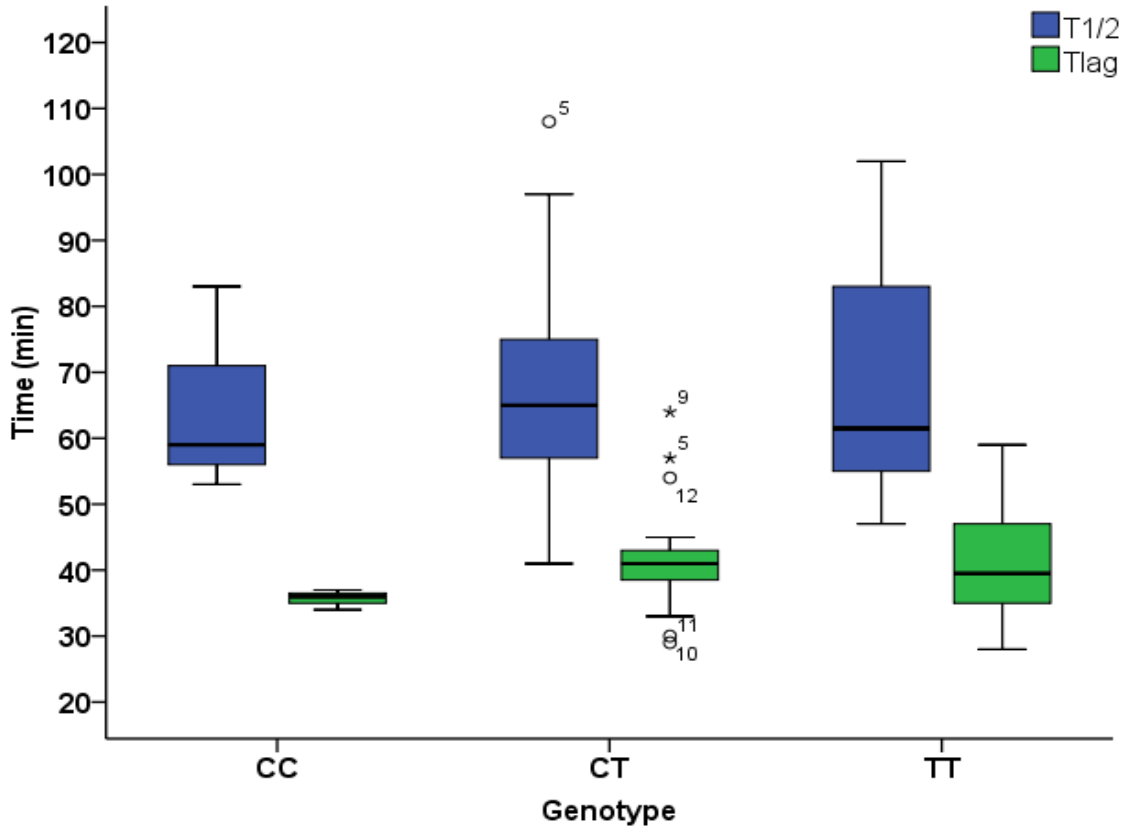

Figure 53. Gastric emptying rate boxplot for SNP 8 rs10305432 according to genotype

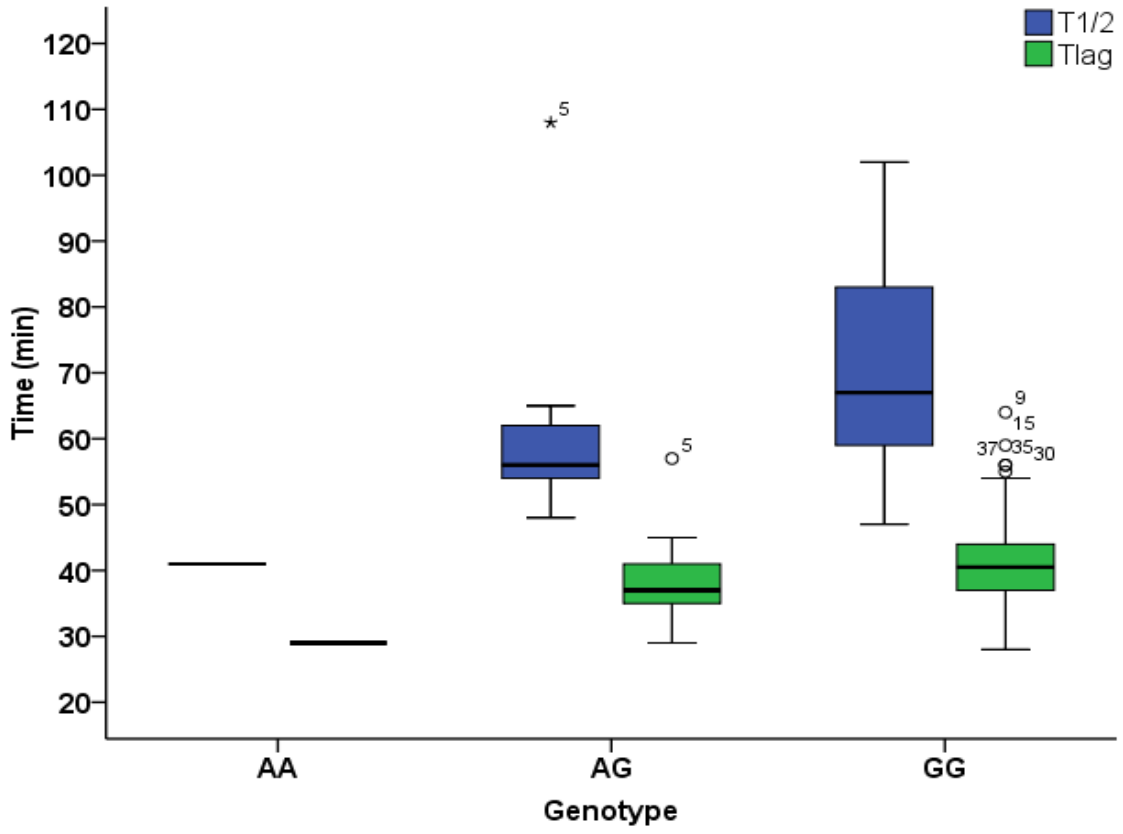

Figure 54. Gastric emptying rate boxplot for SNP 9 rs9283907 according to genotype

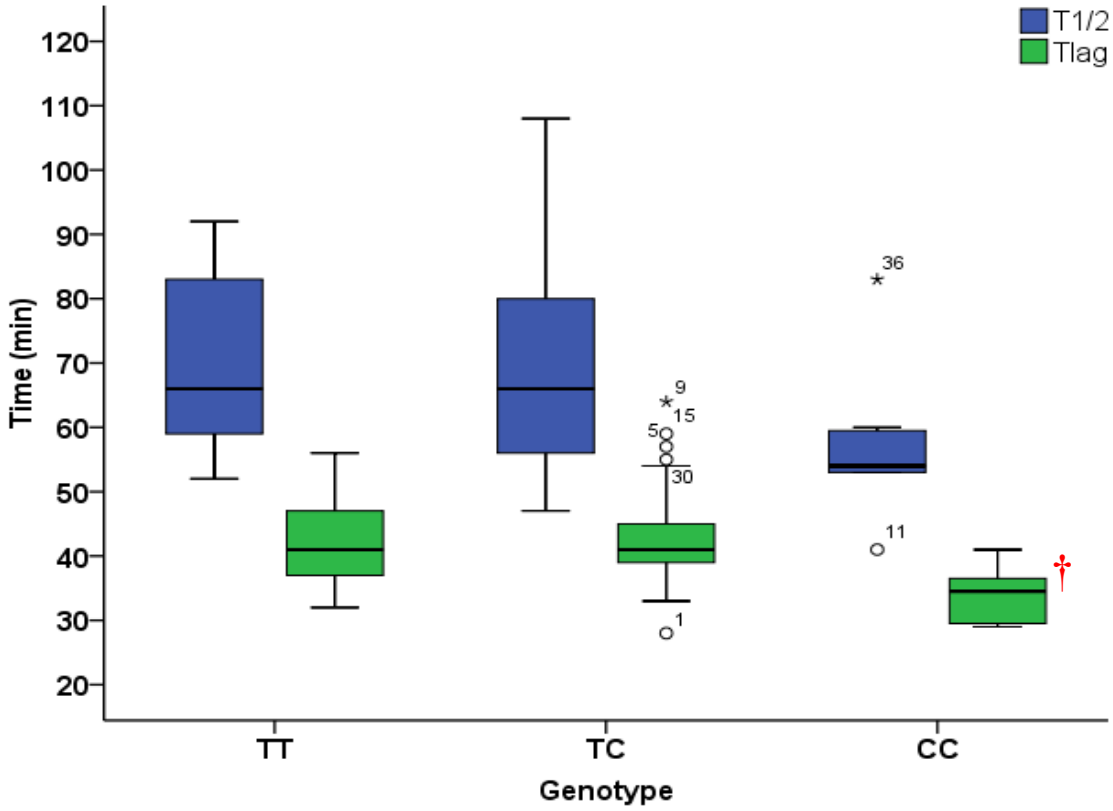

Figure 55. Gastric emptying rate boxplot for SNP 10 rs742764 according to genotype. †CC significantly faster than TT ($P < 0.01$) and TC ($P < 0.01$).

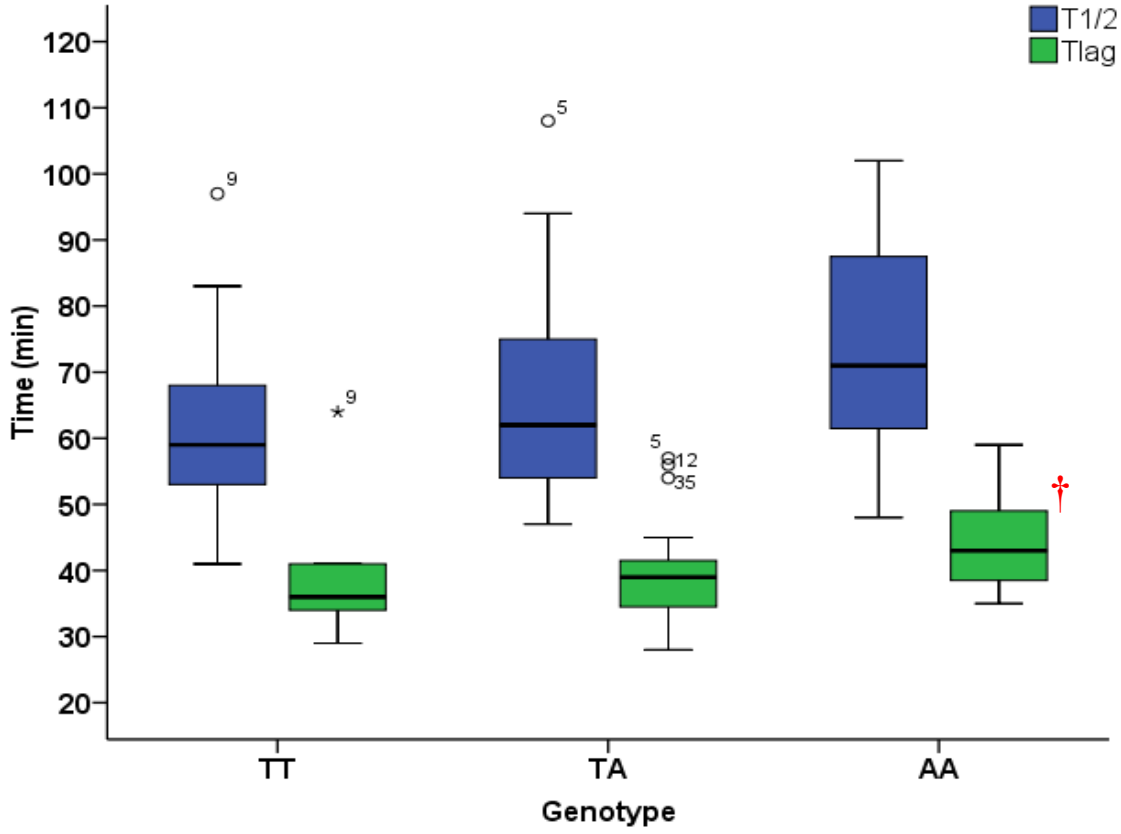

Figure 56. Gastric emptying rate boxplot for SNP 11 rs2254336 according to genotype. †AA significantly slower than TT ($P < 0.05$) and TA ($P < 0.05$).

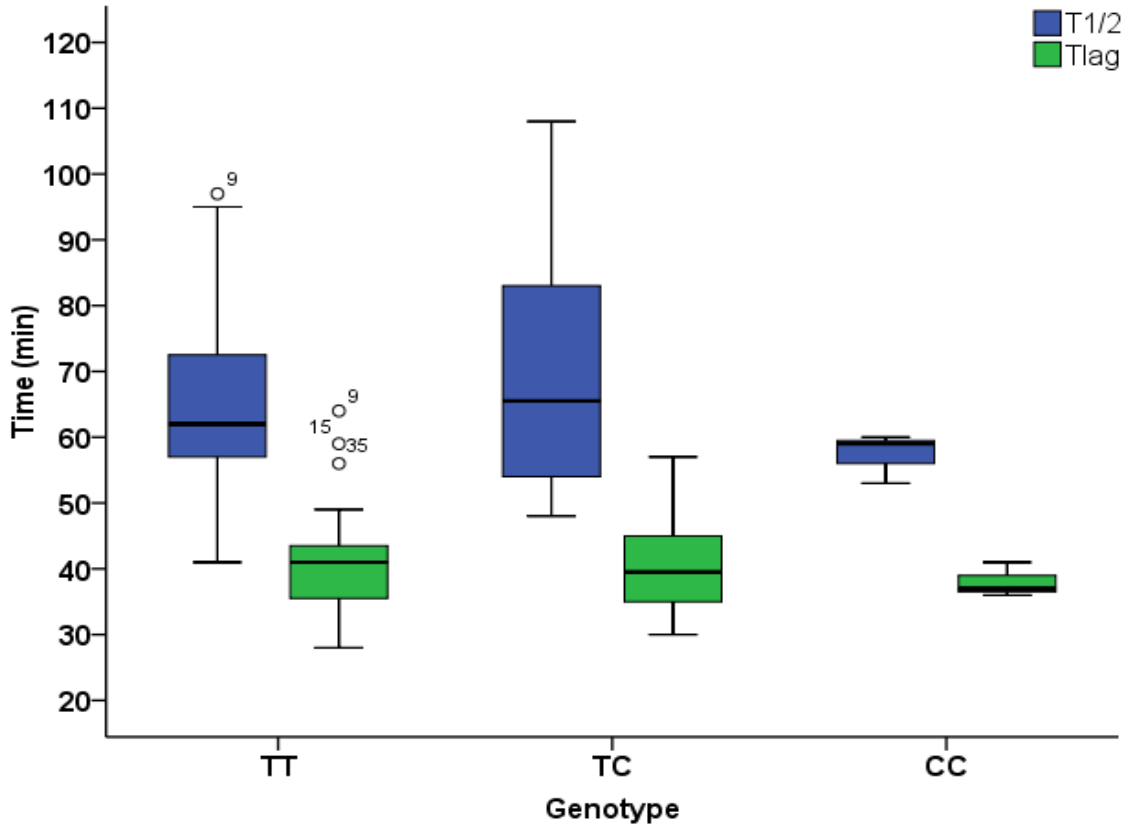

Figure 57. Gastric emptying rate boxplot for SNP 12 rs910163 according to genotype

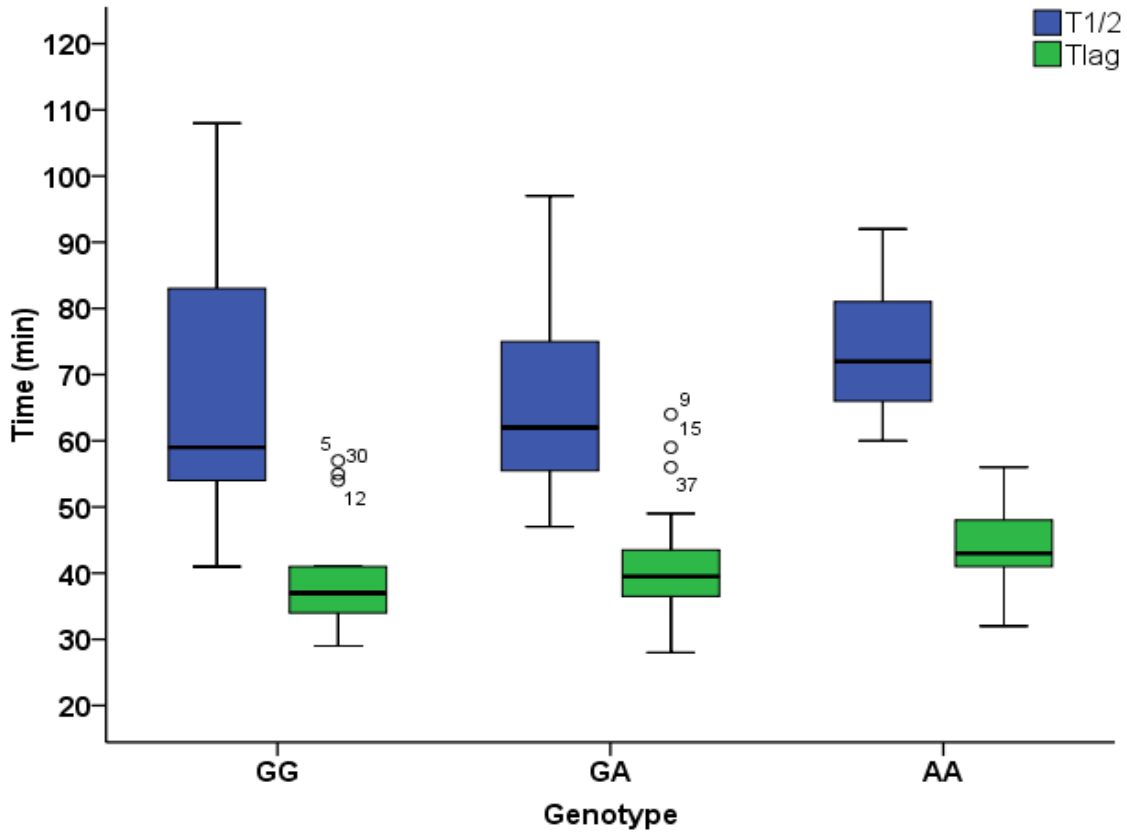

Figure 58. Gastric emptying rate boxplot for SNP 14 rs6923761 according to genotype

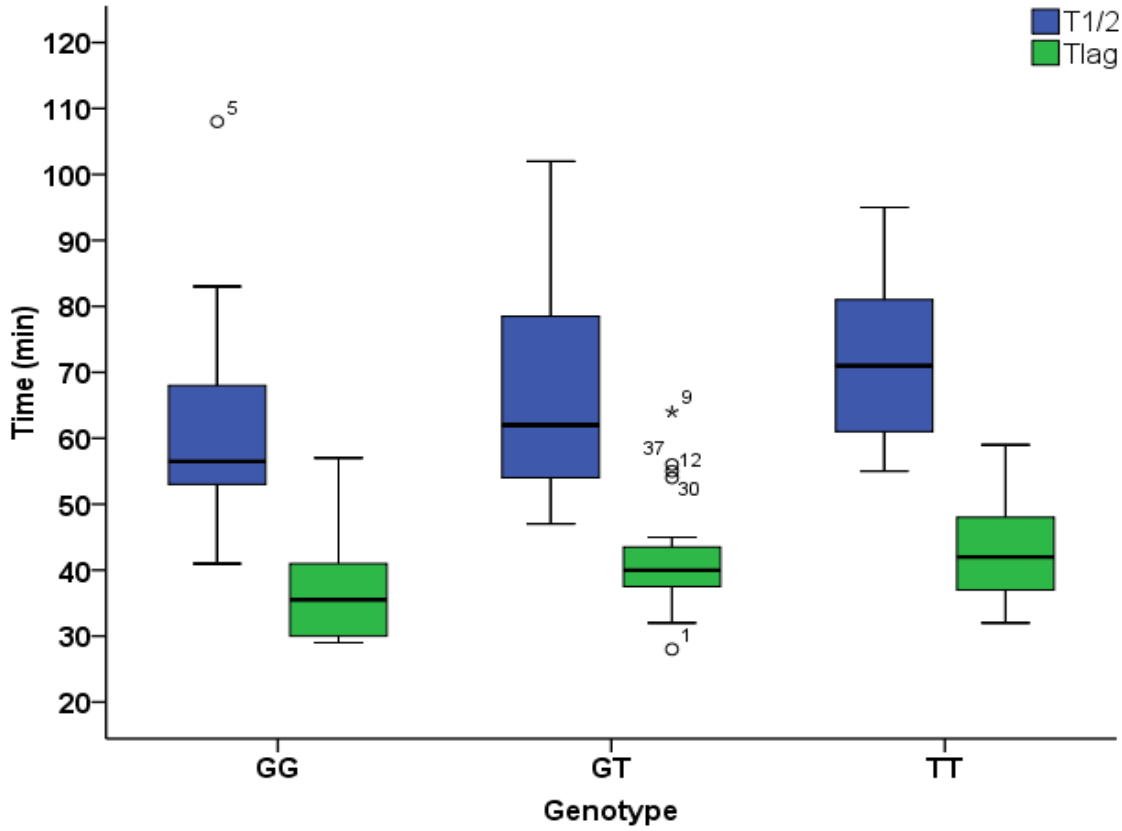

Figure 59. Gastric emptying rate boxplot for SNP 15 rs7766663 according to genotype

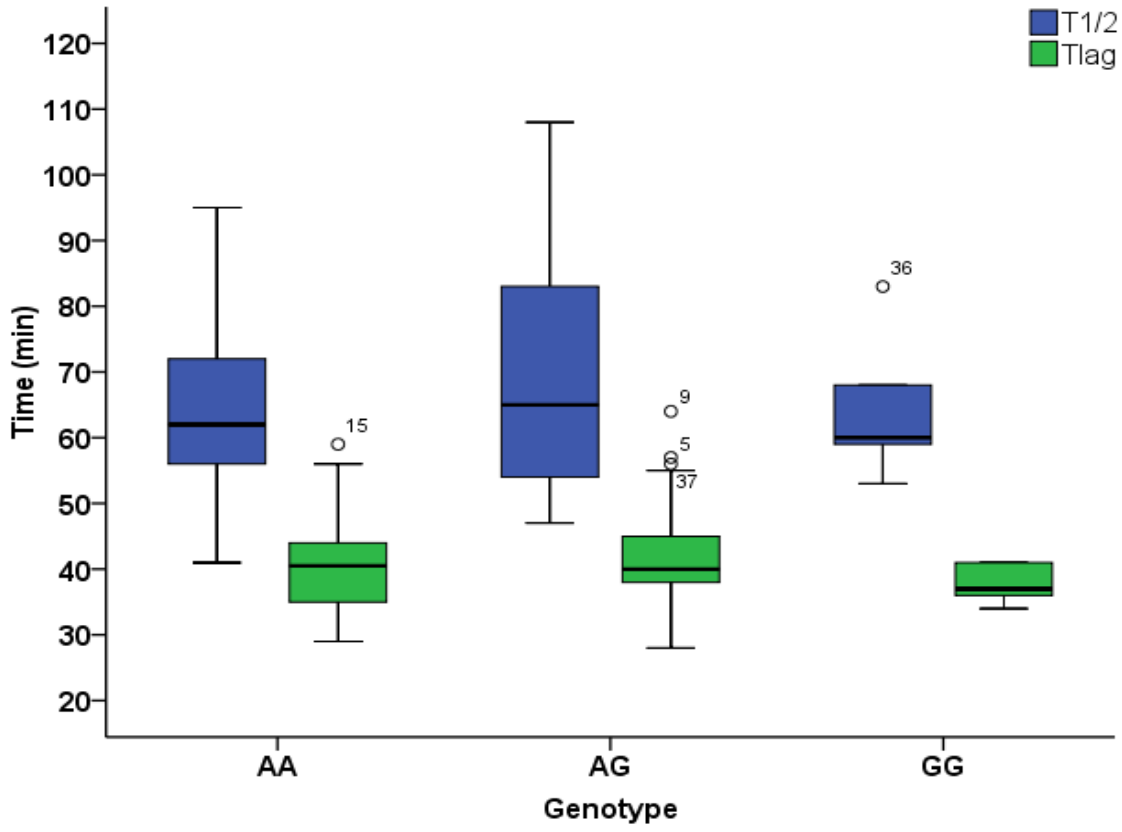

Figure 60. Gastric emptying rate boxplot for SNP 16 rs932443 according to genotype

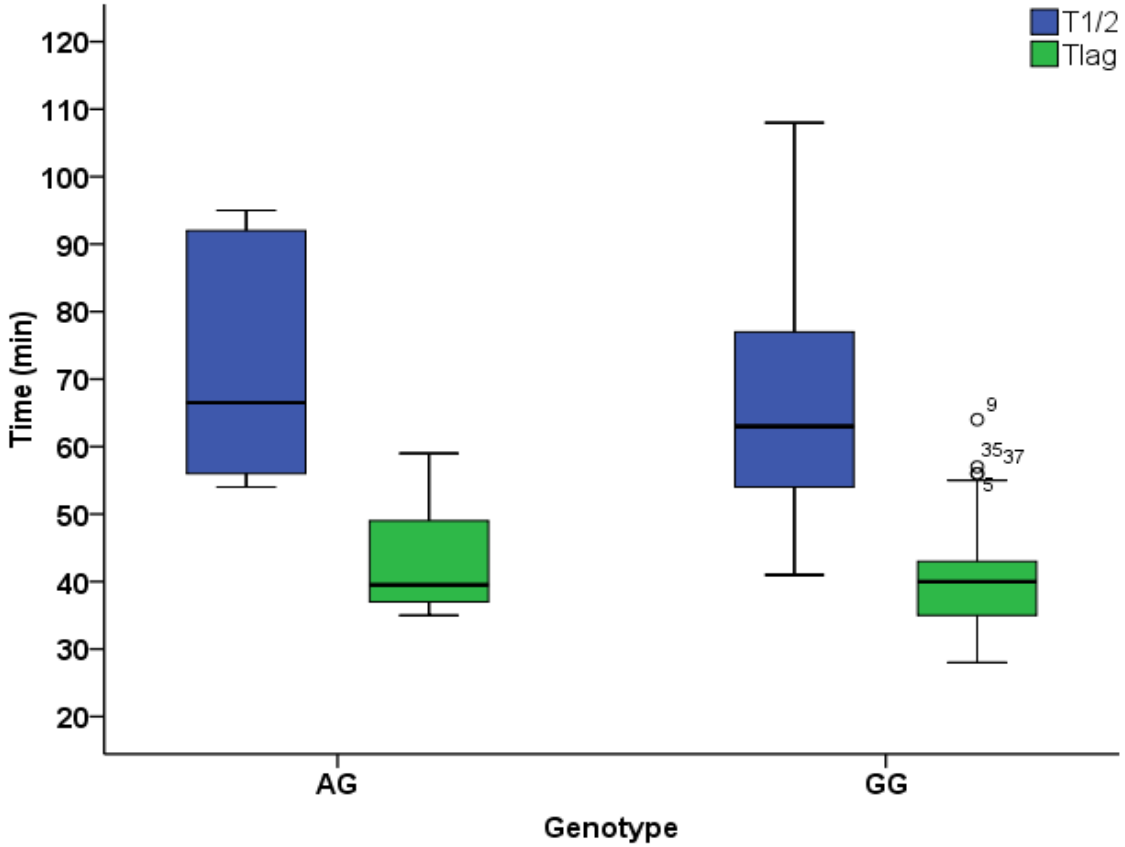

Figure 61. Gastric emptying rate boxplot for SNP 17 rs2268646 according to genotype

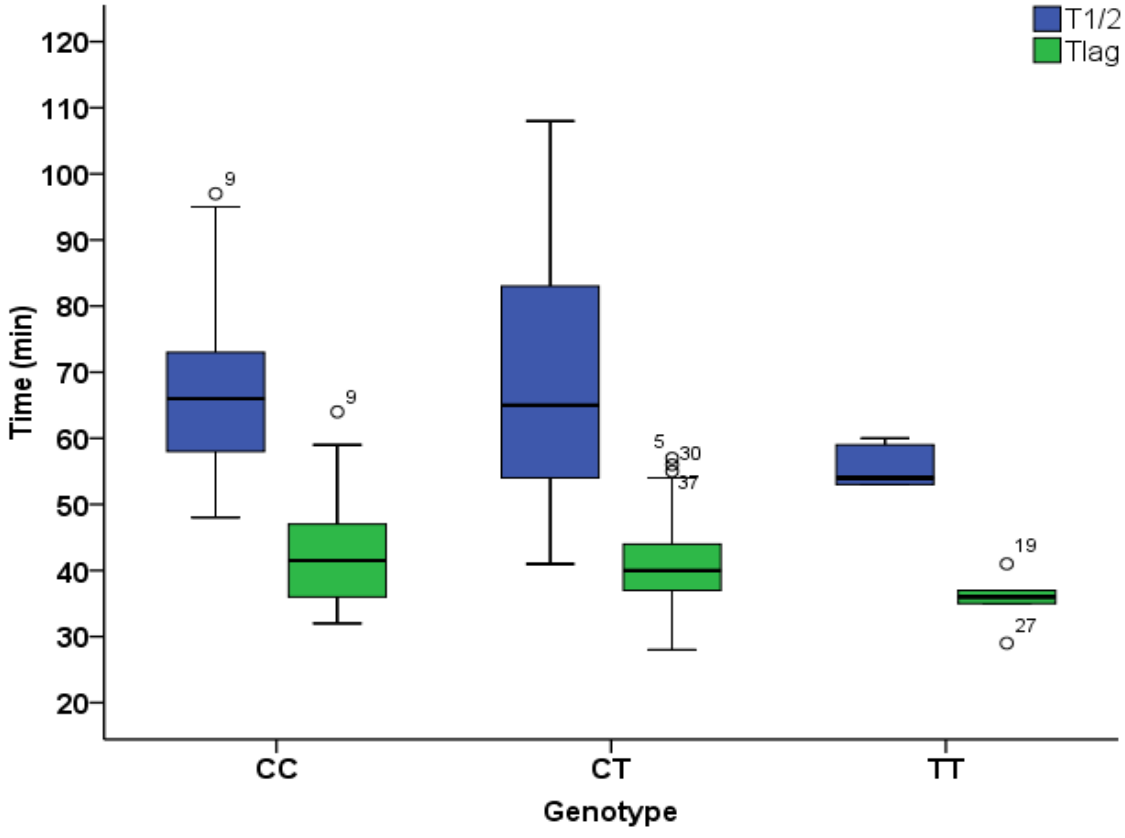

Figure 62. Gastric emptying rate boxplot for SNP 18 rs2300614 according to genotype

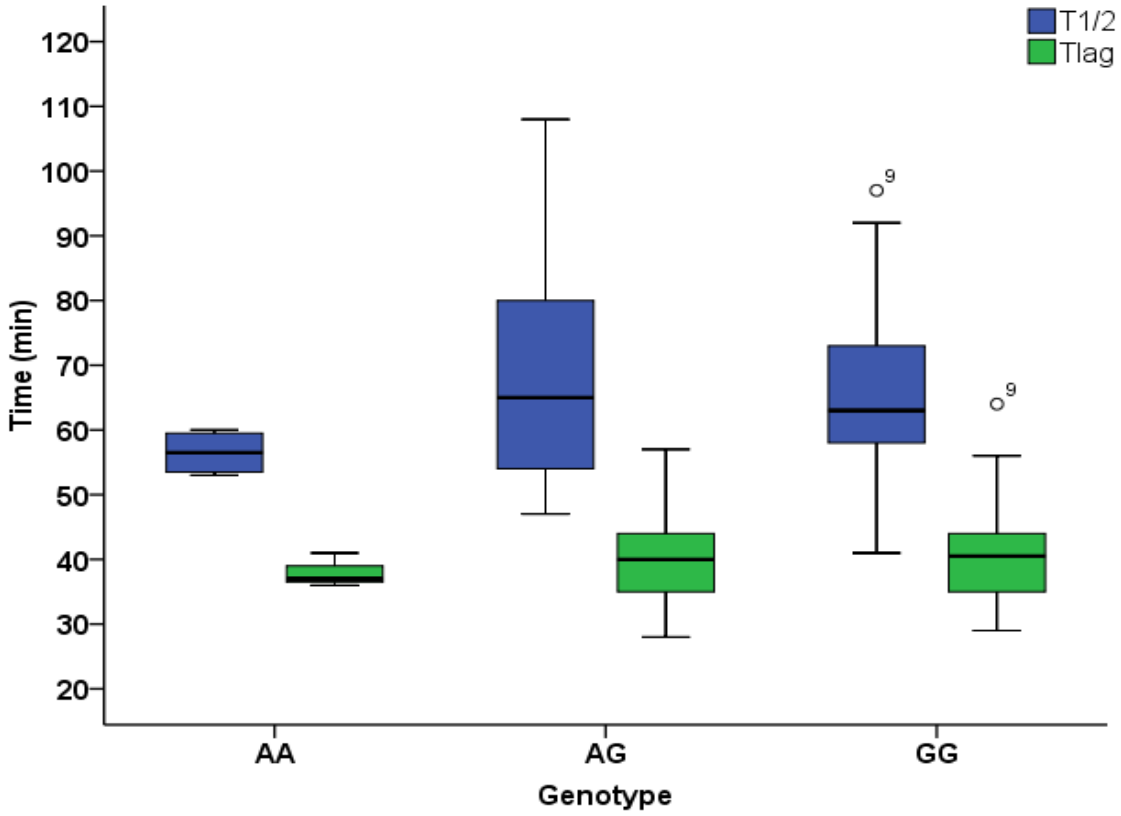

Figure 63. Gastric emptying rate boxplot for SNP 19 rs2268641 according to genotype

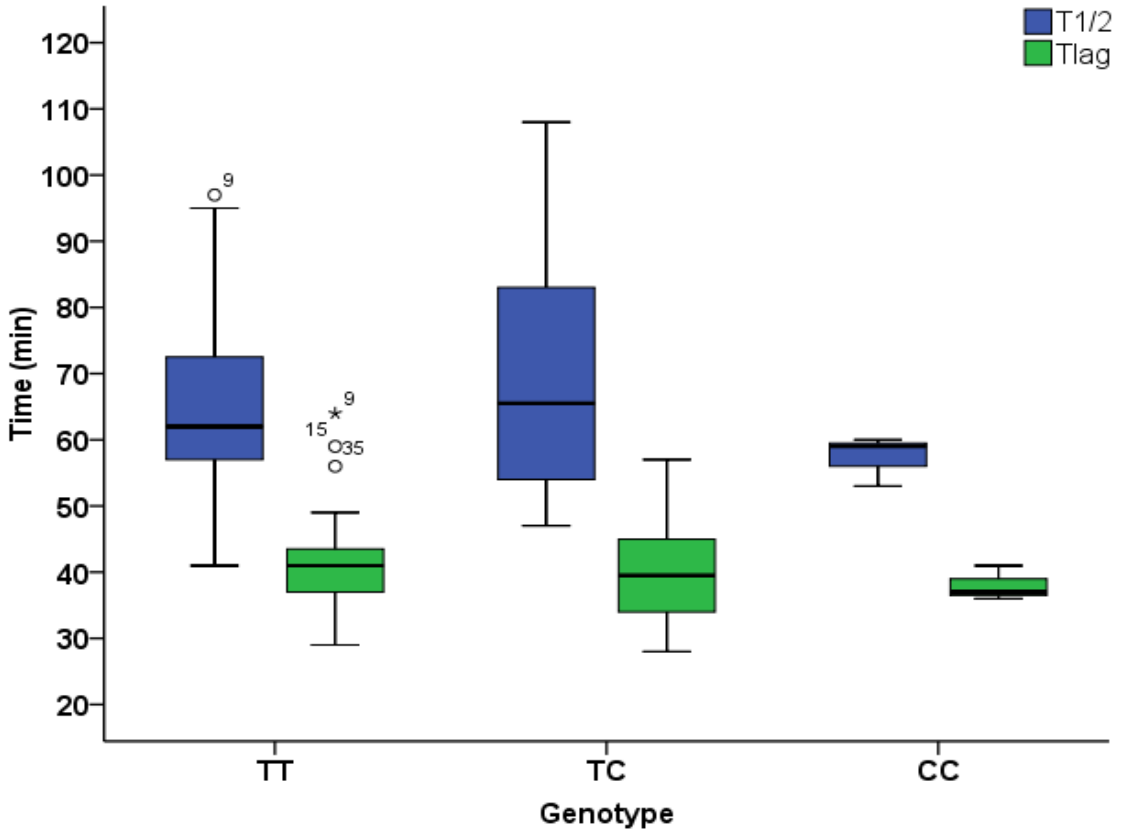

Figure 64. Gastric emptying rate boxplot for SNP 20 rs2268640 according to genotype

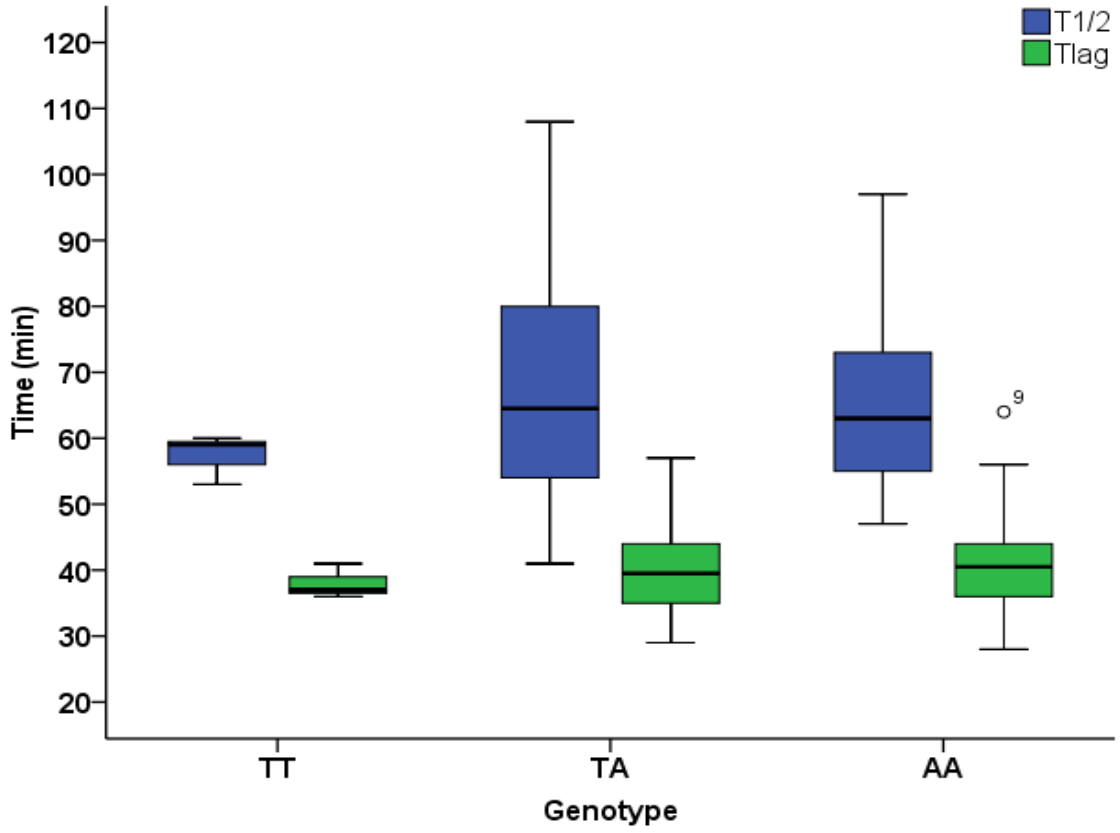

Figure 65. Gastric emptying rate boxplot for SNP 21 rs2268639 according to genotype

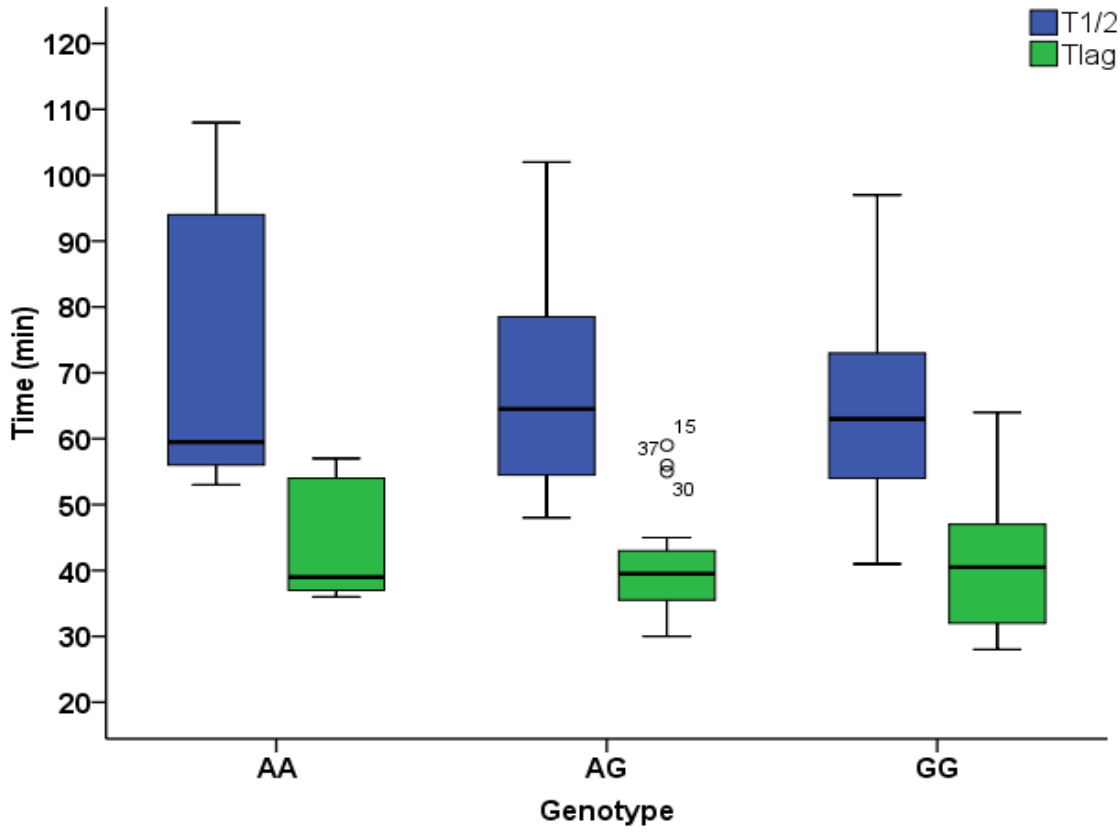

Figure 66. Gastric emptying rate boxplot for SNP 22 rs2206942 according to genotype

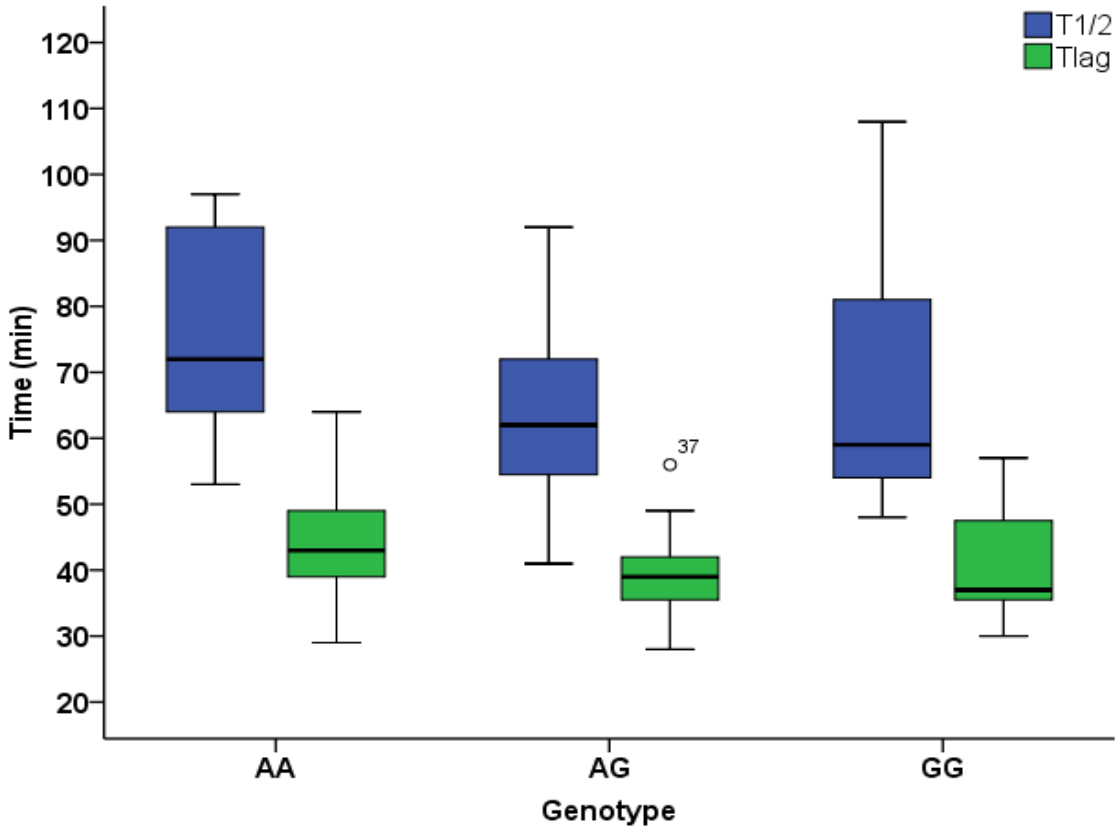

Figure 67. Gastric emptying rate boxplot for SNP 23 rs2894420 according to genotype

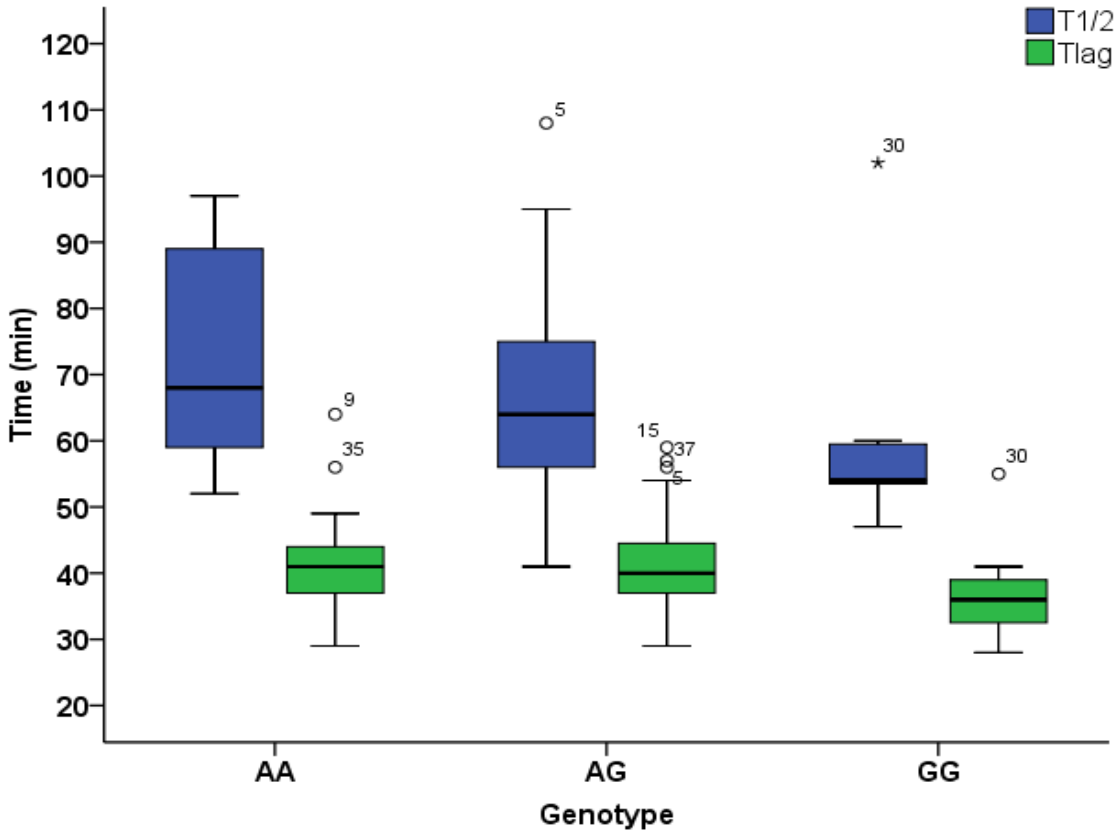

Figure 68. Gastric emptying rate boxplot for SNP 26 rs4714211 according to genotype

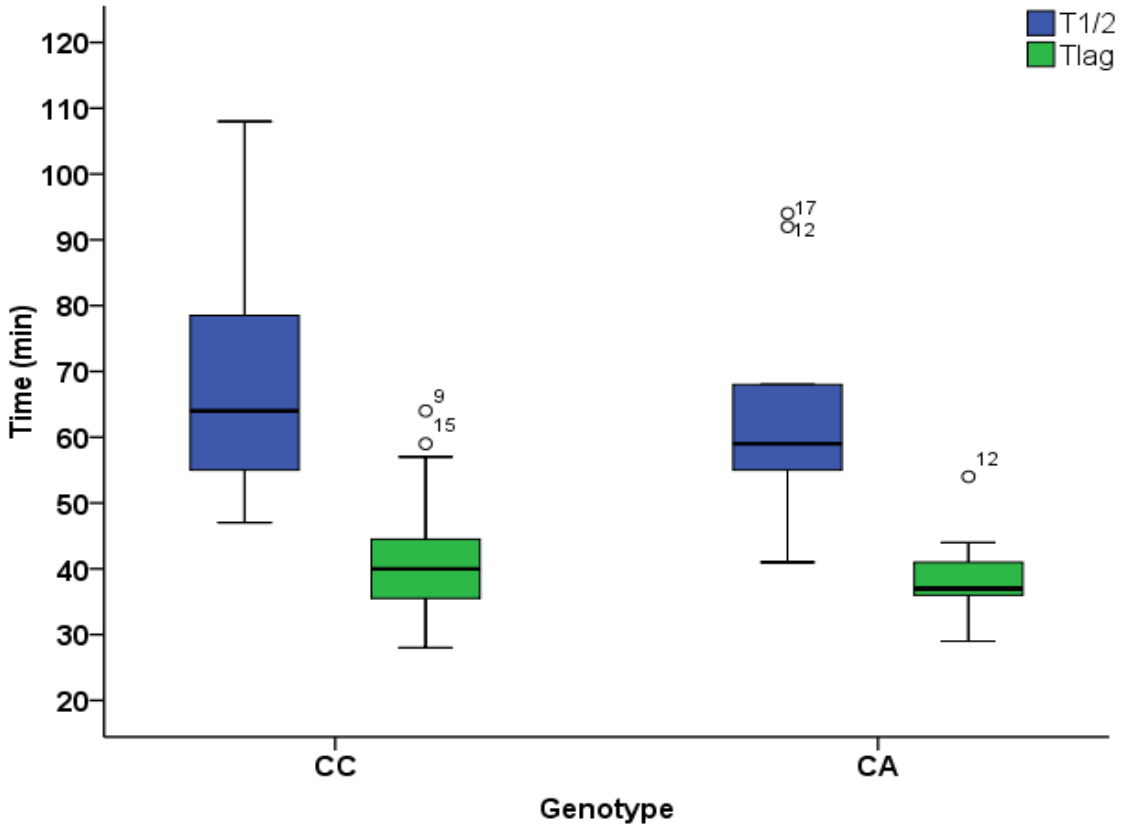

Figure 69. Gastric emptying rate boxplot for SNP 27 rs10305525 according to genotype

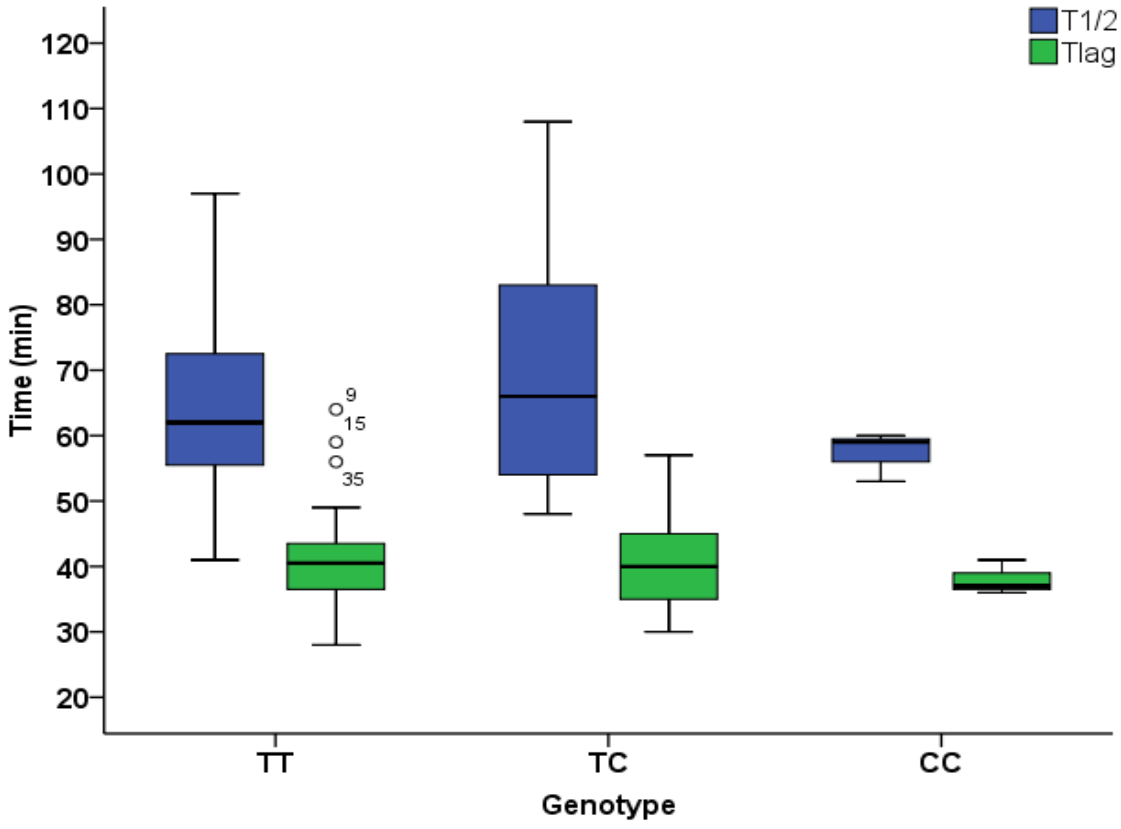

Figure 70. Gastric emptying rate boxplot for SNP 28 rs9296291 according to genotype

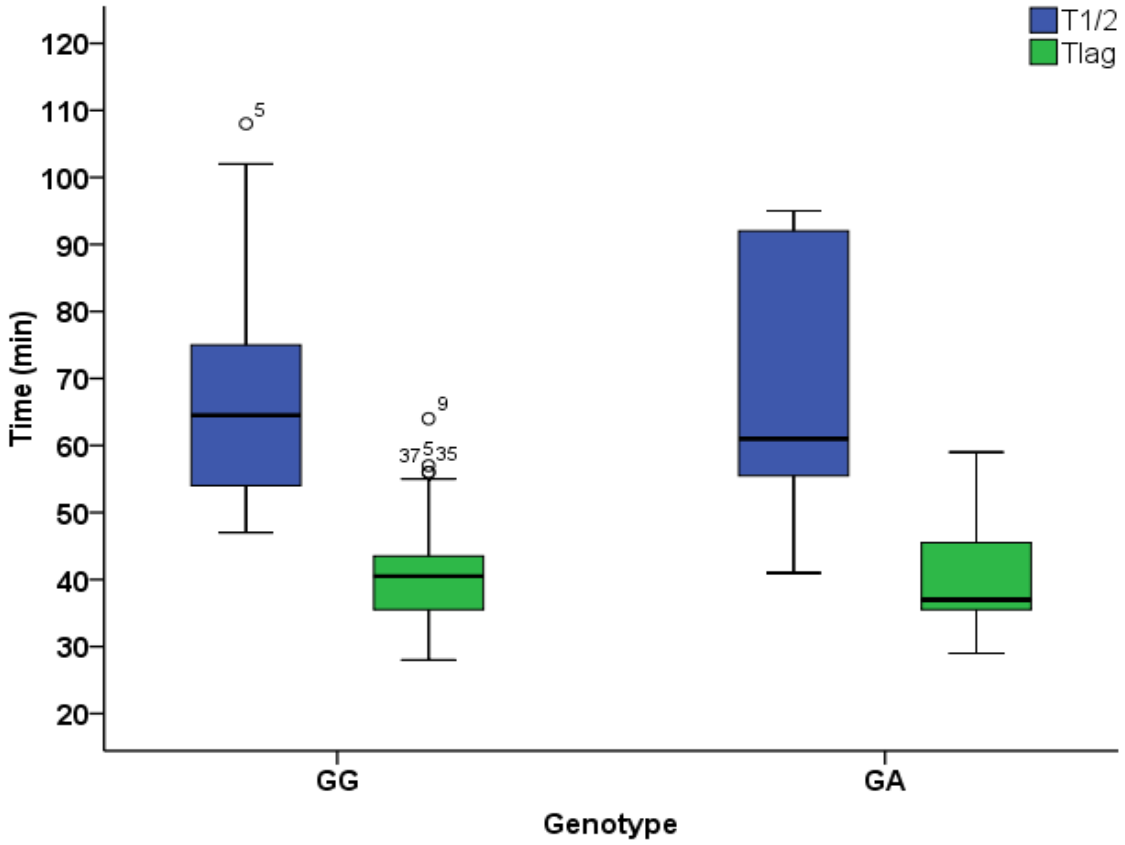

Figure 71. Gastric emptying rate boxplot for SNP 29 rs9968886 according to genotype

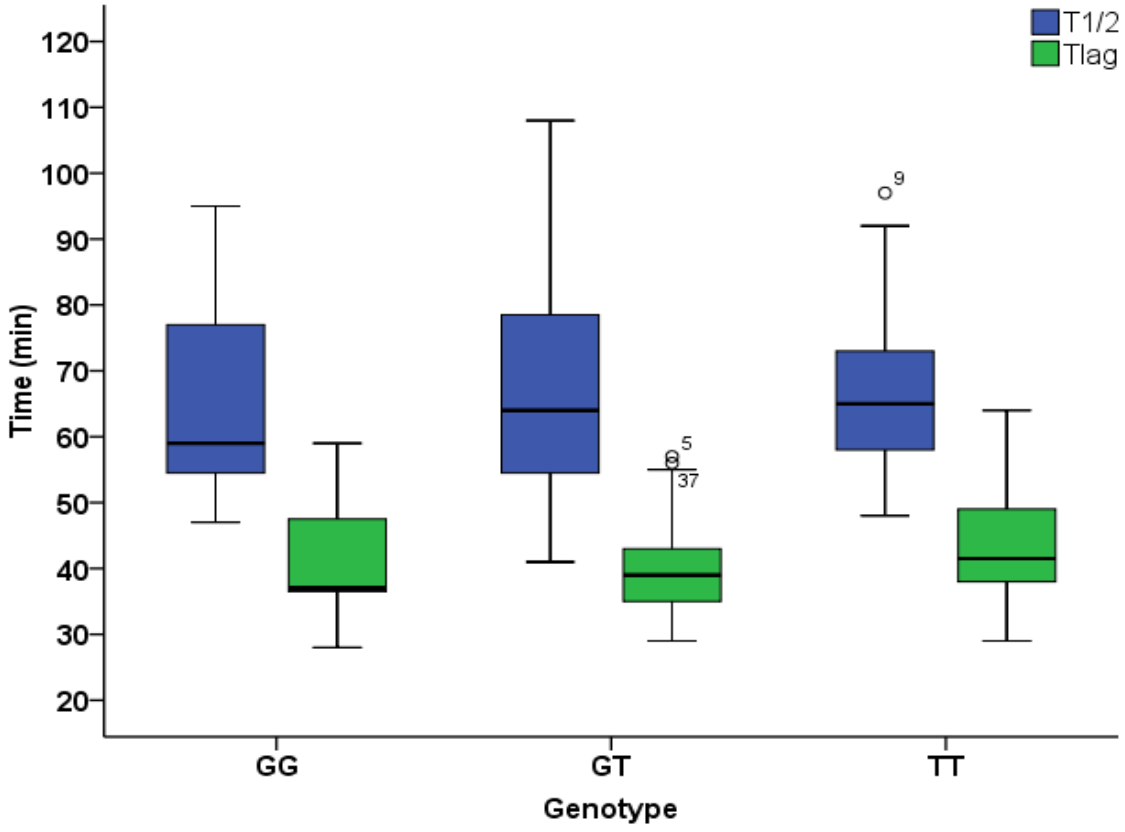

Figure 72. Gastric emptying rate boxplot for SNP 30 rs2143733 according to genotype

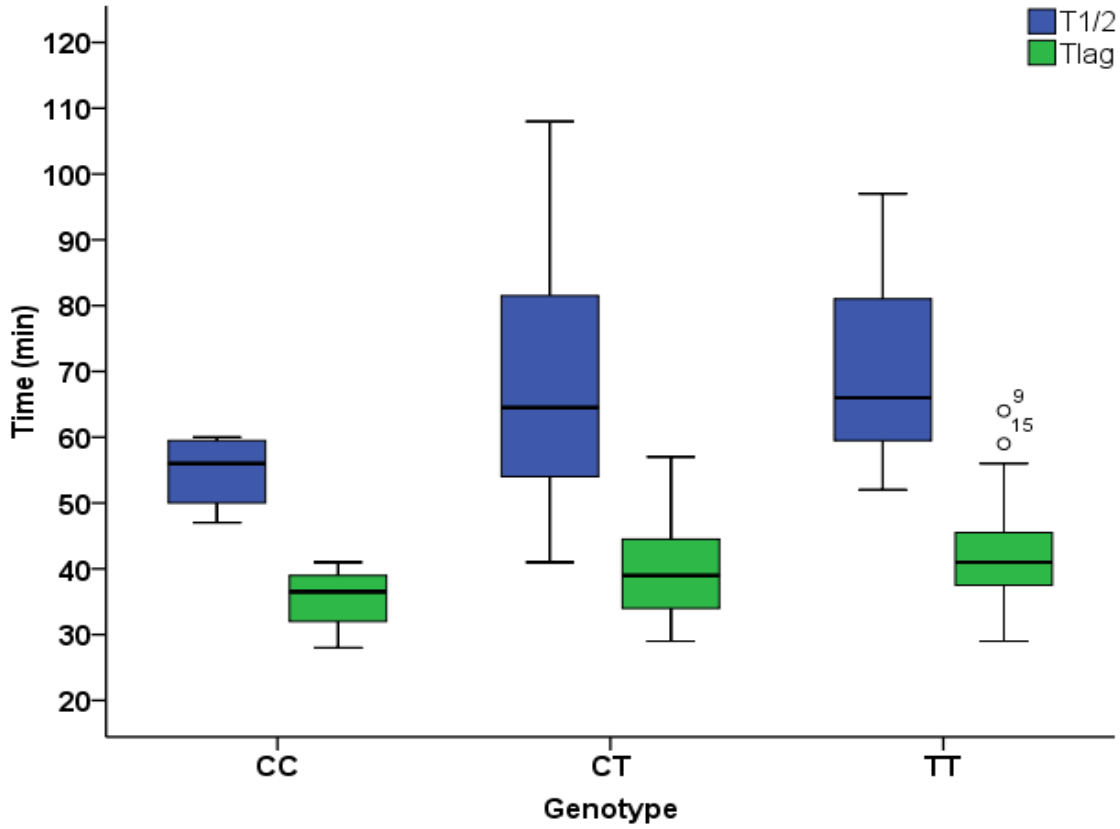

Figure 73. Gastric emptying rate boxplot for SNP 31 rs9296292 according to genotype

6.3.4.2. By phenotype

Results for T_{lag} and $T_{½}$ according to phenotype are shown in Table 9 and Table 10, respectively. Non-parametric statistical analysis revealed a significant effect of the minor allele on median gastric emptying $T_{½}$ for SNP 9 rs9283907, where participants with one or two A alleles had significantly faster $T_{½}$ than participants homozygous for the allele G by 18% (Table 10; $P = 0.033$). A significant effect of the minor allele on median gastric emptying T_{lag} was also seen for SNP 5 rs2268657 and SNP 11 rs2254336. Participants with one or two G alleles had significantly slower T_{lag} than participants homozygous for the allele A by 11% (Table 9; $P = 0.028$), and participants with one or two T alleles had significantly faster T_{lag} than participants homozygous for the allele A by 13% (Table 9; $P = 0.012$), respectively. No significant differences in gastric emptying rate were seen between phenotypes in all other SNPs though $T_{½}$ tended toward significance for SNP 11 rs2254336 ($P = 0.055$) and SNP 15 rs7766663 ($P = 0.097$).

Table 9. Gastric emptying T_{lag} results according to phenotype. Values are minutes.

		Homozygote Major Allele						Heterozygote/homozygote minor allele						
	SNP ID	**Phenotype**	**n**	**Mean**	**SD**	**Median**	**Quartiles**	**Phenotype**	**n**	**Mean**	**SD**	**Median**	**Quartiles**	***P*-value**
1	rs7738586	CC	38	42	8	40	37-45	A	10	40	9	37	34-41	0.274
2	rs9380825	GG	15	39	7	37	36-40	A	33	42	9	41	37-44	0.157
4	rs926674	CC	39	42	9	41	36-46	T	9	38	3	37	36-40	0.239
5	rs2268657	AA	11	36	5	37*	34-39	G	36	42	8	41	37-45	**0.028**
6	rs13202369	AA	25	41	8	39	36-42	G	23	42	9	41	36-48	0.627
7	rs3799707	GG	26	42	8	40	37-45	T	22	40	9	40	34-43	0.396
8	rs10305432	TT	26	41	9	40	35-46	C	22	41	8	41	37-42	0.844
9	rs9283907	GG	38	42	8	41	37-44	A	10	38	8	36	33-41	0.137
10	rs742764	TT	14	42	7	41	37-46	C	33	41	9	40	35-43	0.492
11	rs2254336	AA	16	45	7	43	39-49	T	32	39	8	39*	34-41	**0.012**
12	rs910163	TT	27	41	9	41	36-44	C	21	41	8	39	36-44	0.950
14	rs6923761	GG	13	40	10	37	34-41	A	35	42	8	40	37-44	0.197
15	rs7766663	TT	15	43	8	42	37-48	G	33	40	9	39	35-42	0.136
16	rs932443	AA	22	41	8	41	35-44	G	26	41	9	40	36-44	0.983
17	rs2268646	GG	38	41	8	40	35-43	A	10	43	8	40	37-48	0.493
18	rs2300614	CC	22	42	9	42	36-46	T	26	40	8	39	35-42	0.330
19	rs2268641	GG	22	41	9	41	35-44	A	25	41	8	39	36-42	0.856
20	rs2268640	TT	27	41	8	41	37-44	C	21	41	8	39	35-44	0.546
21	rs2268639	AA	22	41	8	41	36-44	T	25	40	8	39	35-42	0.701
22	rs2206942	GG	18	41	10	41	32-46	A	30	41	8	40	36-44	0.806
23	rs2894420	GG	11	42	9	37	36-48	A	36	40	8	40	36-43	0.930
26	rs4714211	AA	13	43	9	41	37-44	G	34	41	8	40	35-44	0.497
27	rs10305525	CC	39	42	9	40	36-45	A	9	39	7	37	36-41	0.369
28	rs9296291	TT	28	41	9	41	37-43	C	20	41	8	40	35-44	0.908
29	rs9968886	GG	36	41	8	41	36-43	A	12	41	9	37	36-45	0.543
30	rs2143733	TT	14	43	9	42	39-48	G	34	40	8	39	35-44	0.276
31	rs9296292	TT	24	43	8	41	38-45	C	24	40	8	38	34-43	0.107

Reported *P*-values are for median data. *Significantly faster emptying rate compared to other phenotype ($P < 0.05$).

Table 10. Gastric emptying $T_{½}$ according to phenotype. Values are minutes.

		Homozygote Major Allele						Heterozygote/homozygote minor allele						
	SNP ID	**Phenotype**	**n**	**Mean**	**SD**	**Median**	**Quartiles**	**Phenotype**	**n**	**Mean**	**SD**	**Median**	**Quartiles**	***P*-value**
1	rs7738586	CC	38	68	17	64	55-79	A	10	66	15	62	56-72	0.919
2	rs9380825	GG	15	65	15	60	55-70	A	33	69	17	64	56-80	0.367
4	rs926674	CC	39	69	17	64	56-82	T	9	62	10	59	54-65	0.355
5	rs2268657	AA	11	62	14	59	53-67	G	36	69	16	64	56-78	0.119
6	rs13202369	AA	25	68	16	64	56-73	G	23	68	17	62	54-80	0.757
7	rs3799707	GG	26	69	14	66	59-78	T	22	66	19	60	54-76	0.218
8	rs10305432	TT	26	68	16	62	55-80	C	22	68	17	65	55-76	0.836
9	rs9283907	GG	38	70	15	67	59-82	A	10	60	18	55*	53-61	**0.033**
10	rs742764	TT	14	70	14	66	59-81	C	33	67	18	62	54-77	0.478
11	rs2254336	AA	16	73	16	71	62-85	T	32	65	16	60	54-74	0.055
12	rs910163	TT	27	67	15	62	57-73	C	21	69	18	64	54-80	0.950
14	rs6923761	GG	13	68	22	59	54-83	A	35	68	14	64	57-75	0.516
15	rs7766663	TT	15	72	14	71	61-81	G	33	66	17	61	54-77	0.097
16	rs932443	AA	22	66	15	62	57-72	G	26	70	18	65	54-82	0.702
17	rs2268646	GG	38	67	16	63	54-76	A	10	71	17	67	57-87	0.485
18	rs2300614	CC	22	69	15	66	58-73	T	26	67	18	62	54-79	0.521
19	rs2268641	GG	22	68	16	63	58-73	A	25	67	17	62	54-77	0.733
20	rs2268640	TT	27	68	15	62	57-73	C	21	68	18	64	54-80	0.755
21	rs2268639	AA	22	68	18	65	55-79	T	25	67	17	62	54-77	0.806
22	rs2206942	GG	18	67	17	63	55-73	A	30	69	17	63	55-79	0.774
23	rs2894420	GG	11	69	22	59	54-81	A	36	67	14	64	56-74	0.715
26	rs4714211	AA	13	71	16	68	59-89	G	34	66	17	62	54-73	0.385
27	rs10305525	CC	39	69	16	64	55-79	A	9	64	18	59	55-68	0.412
28	rs9296291	TT	28	67	16	62	56-72	C	20	69	18	65	54-81	0.875
29	rs9968886	GG	36	68	16	65	54-74	A	12	69	19	61	56-92	0.934
30	rs2143733	TT	14	69	15	65	59-73	G	34	68	17	63	54-79	0.691
31	rs9296292	TT	24	70	15	66	60-77	C	24	66	18	60	54-78	0.239

Reported *P*-values are for median data. *Significantly faster emptying rate compared to other phenotype ($P < 0.05$).

6.3.4.3. Correlations

No correlations were found between gastric emptying rate T_{lag} and BMI (r = 0.062, *P* = 0.674; Figure 74) or percentage body fat (r = -0.004, *P* = 0.980; Figure 75). In addition, no correlations were found between gastric emptying rate $T_{½}$ and BMI (r =-0.054, *P* =0.716; Figure 76) or percentage body fat (r = -0.079, *P* = 0.593; Figure 77). Furthermore, no correlations were found between gastric emptying rate T_{lag} and BM (r = 0.071, *P* = 0.630; Figure 78) or $T_{½}$ and BM (r = -0.03, *P* = 0.669; Figure 79).

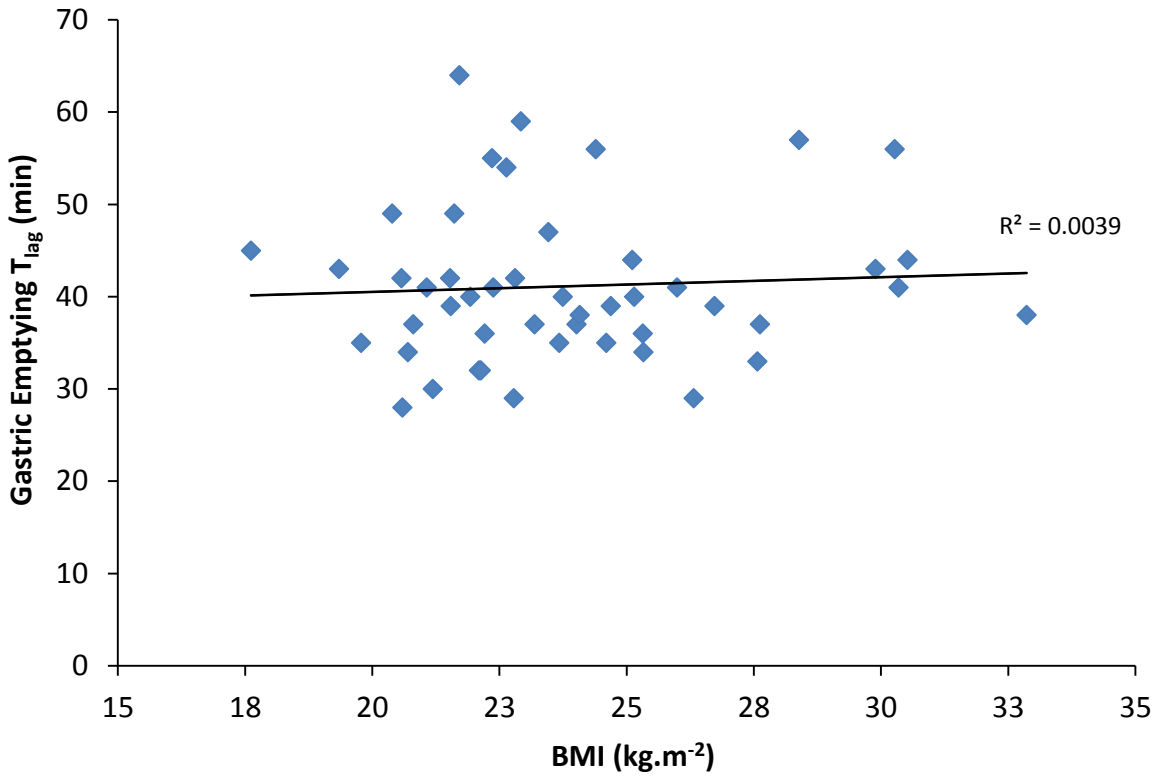

Figure 74.Correlation graph between gastric emptying T_{lag} and body mass index (BMI).

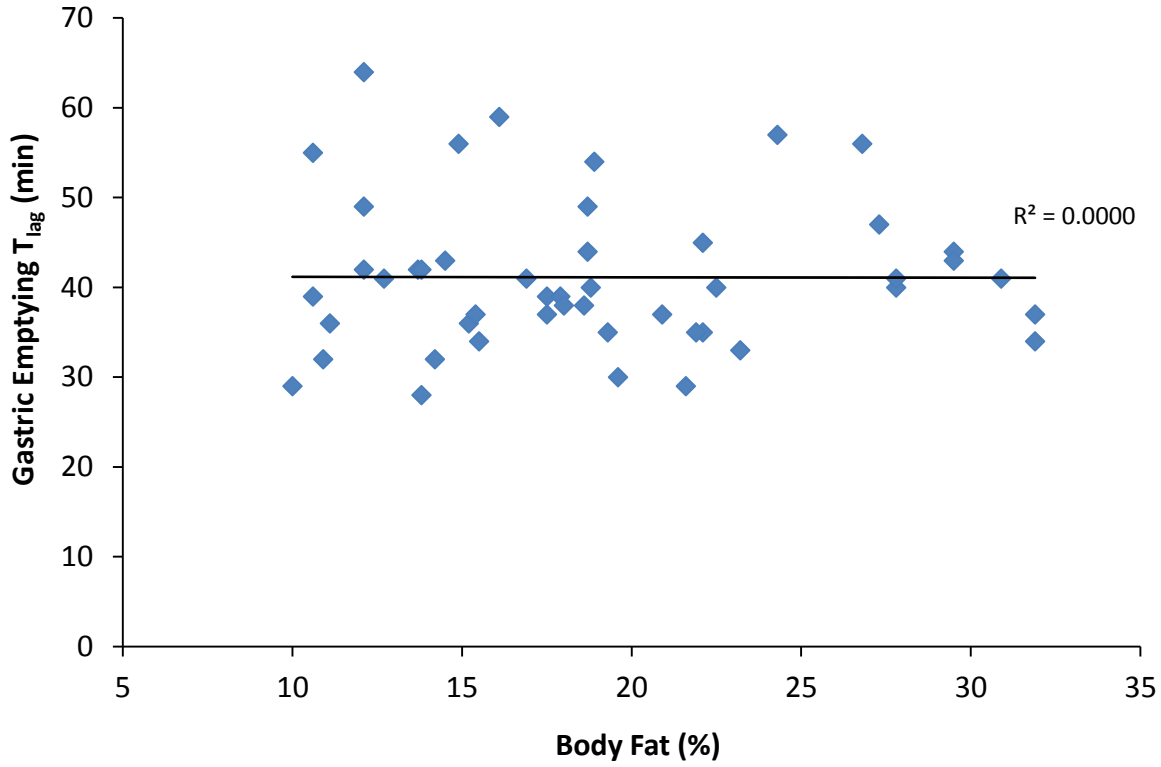

Figure 75. Correlation graph between gastric emptying T_{lag} and body fat percentage

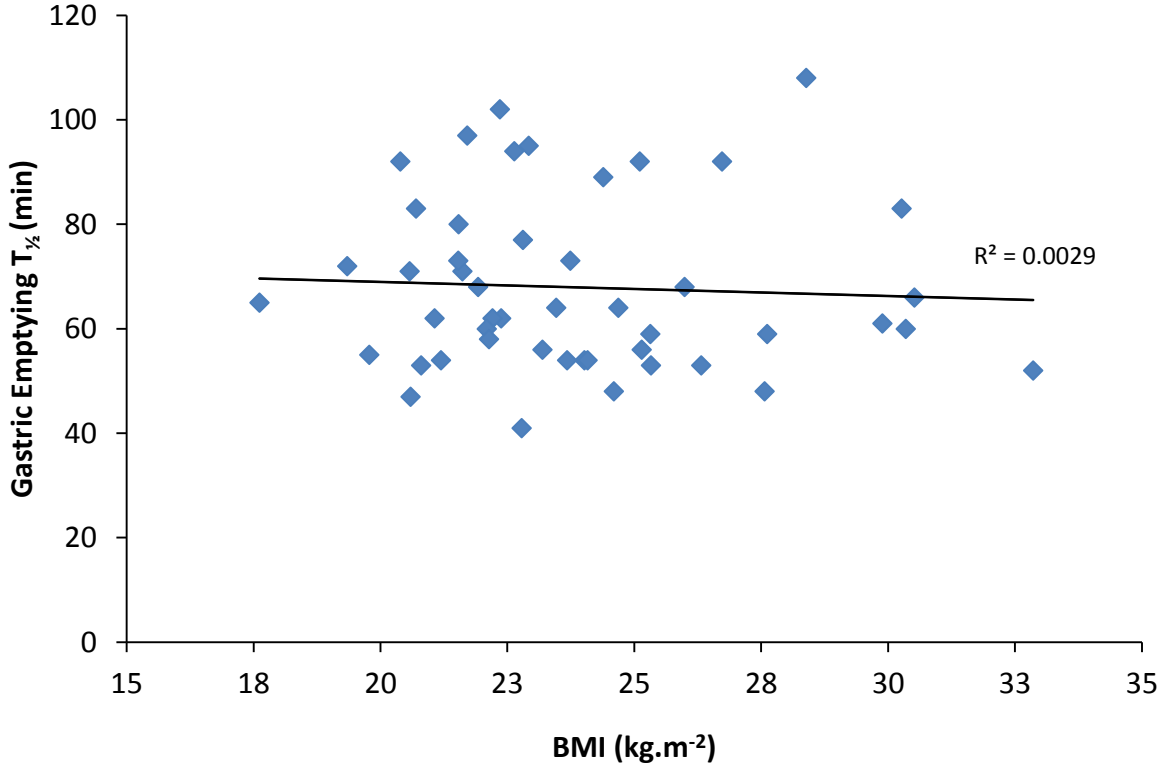

Figure 76. Correlation graph between gastric emptying $T_{½}$ and body mass index (BMI)

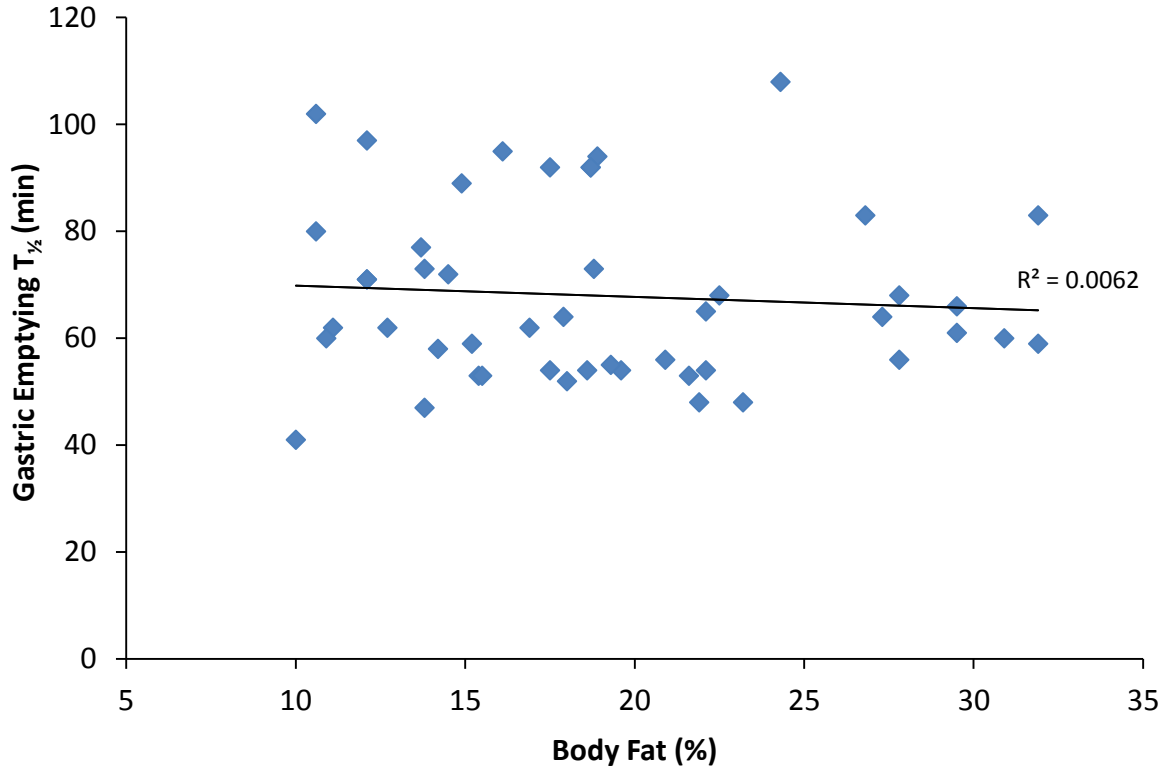

Figure 77. Correlation graph between gastric emptying $T_{½}$ and body fat percentage

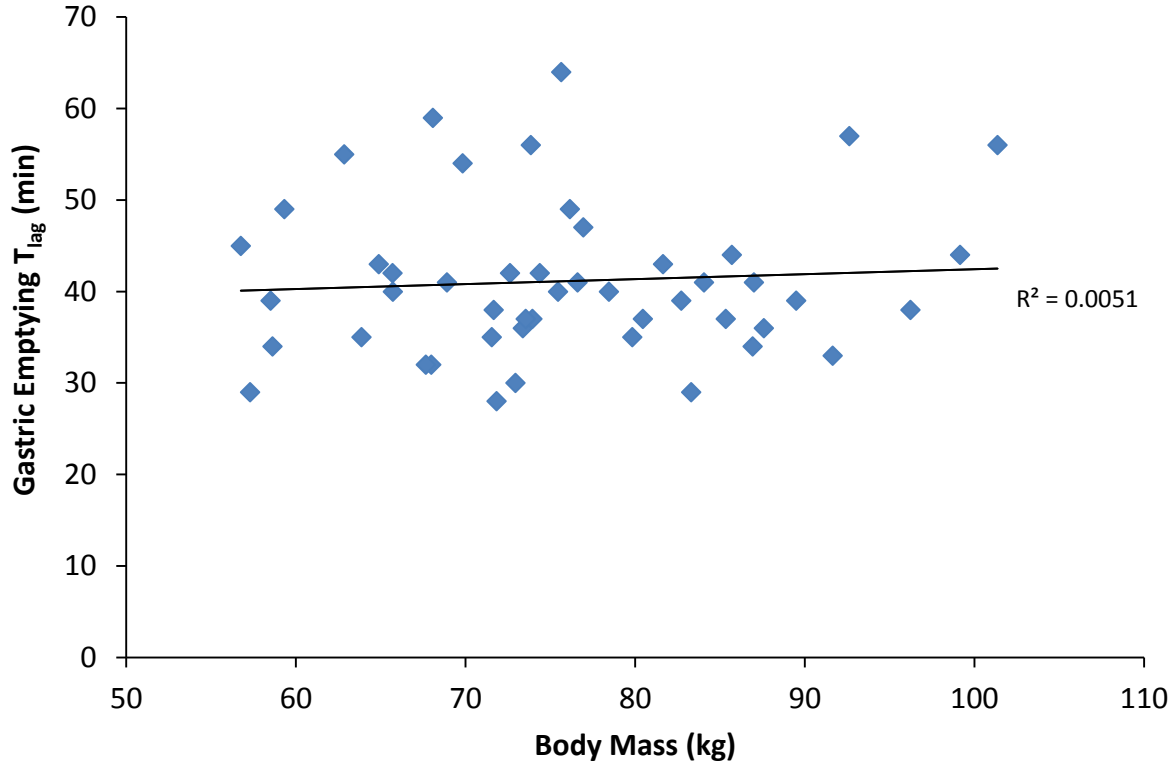

Figure 78. Correlation graph between gastric emptying T_{lag} and body mass

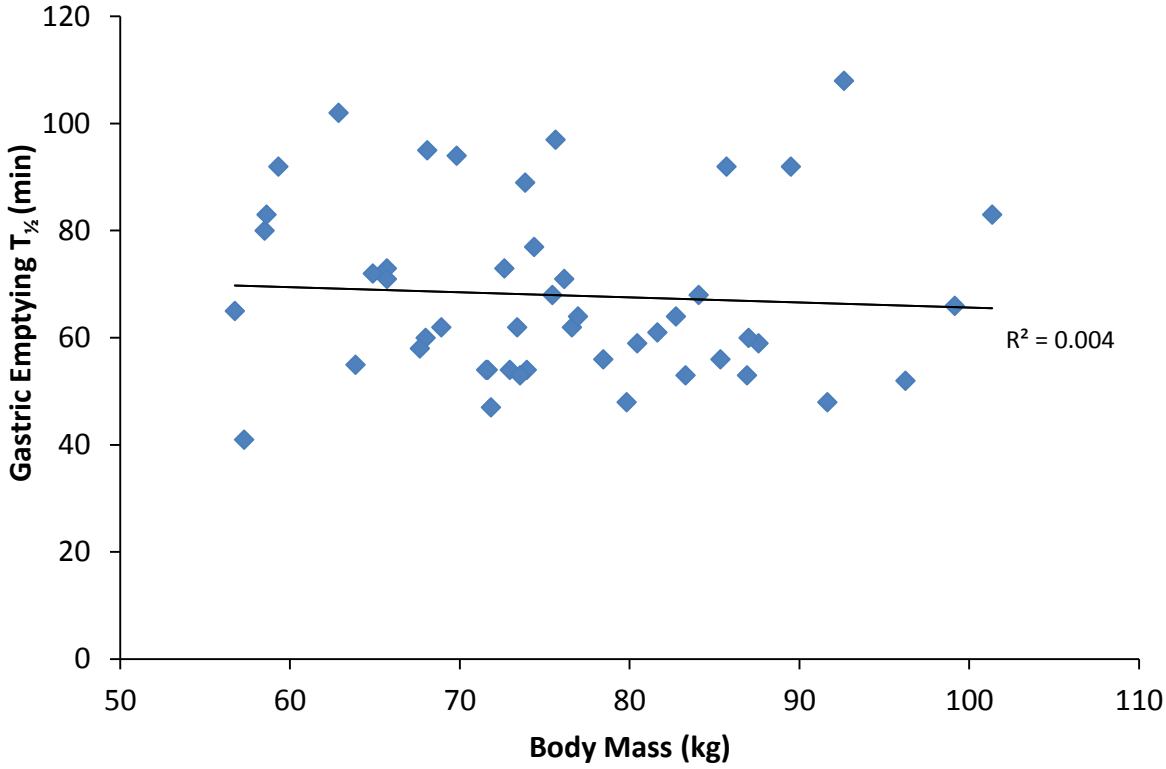

Figure 79. Correlation graph between gastric emptying T½ and body mass

6.3.5. BMI

Mean ± SD BMI for all participants was 23.9 ± 3.3 kg.m^{-2}. Median (quartiles) BMI for all participants was 23.1 (21.6-25.3) kg.m^{-2}. No effect of genotype on BMI were found for all SNPs although SNP 15 tended to significance (P = 0.091). Results by genotype are presented in Table 11. Analysis by phenotype however, revealed significant differences in BMI for SNP 12 rs910163 (P = 0.039) and SNP 15 rs7766663 (P = 0.028) (Table 12). Median BMI was significantly higher in participants with one or two minor alleles compared to homozygotes of the major allele for both SNPs.

6.3.6. Body fat percentage

Mean ± SD body fat for all participants was 19.0 ± 6.2%. Median (quartiles) body fat for all participants was 18.3 (14.1-22.2)%. No effect of genotype nor phenotype was seen for any SNPs although SNP 28 tended to significance for phenotype (P = 0.061). Results by genotype are presented in Table 13 and results by phenotype are presented in Table 14.

Table 11. Body mass index according to genotype. Values are $kg.m^{-2}$.

		Homozygous Minor Allele					Heterozygous					Homozygous Major Allele					
	SNP ID	**Genotype**	**Mean**	**SD**	**Median**	**Quartiles**	**Genotype**	**Mean**	**SD**	**Median**	**Quartiles**	**Genotype**	**Mean**	**SD**	**Median**	**Quartiles**	***P*-value**
1	rs7738586	---	--	--	--	--	CA	22.7	2.5	21.9	21.2-23.6	CC	24.2	3.4	23.6	22.0-25.3	0.141
2	rs9380825	AA	25.5	4.5	25.3	21.5-29.9	AG	23.8	2.8	23.1	21.9-24.8	GG	23.6	3.7	22.9	21.2-25.7	0.751
4	rs926674	TT	21.2	3.9	20.8	19.2-23.1	TC	23.7	1.6	20.5	18.1-26.4	CC	24.1	3.4	22.8	21.7-26.2	0.463
5	rs2268657	GG	24.0	3.5	24.4	21.5-25.3	AG	23.8	3.0	22.8	21.8-24.6	AA	24.0	4.3	24.0	20.8-26.4	0.994
6	rs13202369	GG	23.7	4.3	23.7	22.1-25.2	AG	24.9	3.8	23.2	22.4-27.6	AA	23.0	2.6	22.8	21.1-25.1	0.319
7	rs3799707	TT	25.6	4.7	26.3	23.4-28.1	GT	24.0	2.7	23.7	22.2-25.1	GG	23.6	3.6	22.8	21.5-25.2	0.635
8	rs10305432	CC	22.3	2.6	20.8	20.8-23.1	CT	24.4	3.9	22.8	21.6-26.8	TT	23.7	2.9	23.3	22.0-25.1	0.565
9	rs9283907	AA	22.8	--	22.8	22.8-22.8	AG	23.8	3.0	24.0	22.4-25.1	GG	23.9	3.4	22.9	21.5-25.3	0.830
10	rs742764	CC	23.9	3.3	23.2	21.1-25.6	TC	23.9	3.1	23.7	21.7-25.3	TT	23.9	4.0	22.7	21.6-24.9	0.833
11	rs2254336	TT	24.2	3.1	23.7	21.7-26.0	TA	24.0	3.5	23.7	21.8-25.2	AA	23.6	3.2	22.6	21.6-24.7	0.755
12	rs910163	CC	25.5	4.8	25.3	23.1-27.8	TC	24.6	3.2	24.3	22.7-26.4	TT	23.2	3.1	22.2	21.3-24.2	0.117
14	rs6923761	AA	21.9	1.7	21.9	21.0-22.8	GA	24.2	3.6	23.5	21.7-25.7	GG	24.2	3.0	23.7	22.4-26.0	0.150
15	rs7766663	GG	24.6	3.3	24.5	21.6-26.2	GT	24.5	3.7	24.0	22.2-26.0	TT	22.4	2.2	22.1	21.1-23.3	0.091
16	rs932443	GG	24.6	4.0	25.3	20.8-26.0	AG	24.4	3.7	23.7	21.7-26.3	AA	23.2	2.6	22.6	21.7-24.3	0.405
17	rs2268646	--	--	--	--	--	AG	23.0	2.3	22.8	21.8-23.8	GG	24.1	3.5	23.6	21.6-25.8	0.431
18	rs2300614	TT	25.3	3.5	25.3	23.7-26.3	CT	23.7	3.2	22.8	21.5-24.7	CC	23.7	3.4	22.6	21.6-25.3	0.504
19	rs2268641	AA	25.1	4.0	24.7	23.2-26.6	AG	23.6	3.2	23.2	21.2-24.7	GG	24.0	3.4	22.6	21.8-26.2	0.729
20	rs2268640	CC	25.5	4.8	25.3	23.1-27.8	TC	24.1	3.4	23.7	21.7-25.3	TT	23.6	3.1	22.8	21.7-24.7	0.608
21	rs2268639	TT	25.5	4.8	25.3	23.1-27.8	TA	23.6	3.1	23.0	21.7-24.7	AA	24.0	3.5	22.9	21.6-26.2	0.783
22	rs2206942	AA	25.1	3.6	24.3	22.8-27.6	AG	24.0	3.6	23.7	21.5-25.2	GG	23.3	2.8	22.3	21.6-24.3	0.431
23	rs2894420	AA	22.9	2.5	22.1	21.7-24.4	AG	24.0	3.7	22.8	21.5-25.2	GG	24.4	3.0	23.7	22.5-25.7	0.548
26	rs4714211	GG	23.5	3.5	22.4	21.0-24.5	AG	24.3	3.1	23.7	22.4-25.7	AA	23.4	3.8	22.2	20.6-25.1	0.342
27	rs10305525	--	--	--	--	--	CA	24.7	3.8	23.2	22.6-26.0	CC	23.7	3.2	22.9	21.5-25.2	0.420
28	rs9296291	CC	25.5	4.8	25.3	23.1-27.8	TC	24.3	3.4	23.7	22.4-25.3	TT	23.5	3.1	22.6	21.6-24.6	0.420
29	rs9968886	--	--	--	--		GA	23.5	2.1	23.1	22.5-24.3	GG	24.0	3.6	23.2	21.4-25.5	0.886
30	rs2143733	GG	23.7	3.3	22.9	21.7-24.3	GT	24.3	3.5	23.7	22.2-25.7	TT	23.2	3.0	22.0	21.6-24.3	0.429
31	rs9296292	CC	24.3	4.6	23.1	20.8-26.6	CT	24.0	3.2	23.4	22.3-25.2	TT	23.7	3.3	22.6	21.6-25.3	0.760

Reported *P*-values are for median data.

Table 12. Body mass index according to phenotype. Values are $kg.m^{-2}$.

		Homozygote Major Allele						Heterozygote/homozygote minor allele						
	SNP ID	**Phenotype**	**n**	**Mean**	**SD**	**Median**	**Quartiles**	**Phenotype**	**n**	**Mean**	**SD**	**Median**	**Quartiles**	***P*-value**
1	rs7738586	CC	38	24.2	3.4	23.6	22.0-25.3	A	10	22.7	2.5	21.9	21.2-23.6	0.141
2	rs9380825	GG	15	23.6	3.7	22.9	21.2-25.7	A	33	24.0	3.1	23.5	21.7-25.1	0.648
4	rs926674	CC	39	24.1	3.4	22.8	21.7-26.2	T	9	22.9	2.6	23.7	20.8-24.7	0.663
5	rs2268657	AA	11	24.0	4.3	24.0	20.8-26.4	G	36	23.9	3.0	23.0	21.7-25.1	1.000
6	rs13202369	AA	25	23.0	2.6	22.8	21.1-25.1	G	23	24.8	3.7	23.2	22.2-27.1	0.151
7	rs3799707	GG	26	23.6	3.6	22.8	21.5-25.2	T	22	24.2	2.9	23.9	22.2-25.3	0.385
8	rs10305432	TT	26	23.7	2.9	23.3	22.0-25.1	C	22	24.1	3.8	22.8	21.5-25.8	0.852
9	rs9283907	GG	38	23.9	3.4	22.9	21.5-25.3	A	10	23.7	2.9	23.8	22.5-25.0	0.612
10	rs742764	TT	14	23.9	4.0	22.7	21.6-24.9	C	33	23.9	3.1	23.7	21.6-25.3	0.577
11	rs2254336	AA	16	23.6	3.2	22.6	21.6-24.7	T	32	24.0	3.4	23.7	21.7-25.5	0.470
12	rs910163	TT	27	23.2	3.1	22.2	21.3-24.2	C	21	24.7	3.4	24.6*	22.6-26.7	**0.039**
14	rs6923761	GG	13	24.2	3.0	23.7	22.4-26.0	A	35	23.8	3.4	22.9	21.6-25.1	0.539
15	rs7766663	TT	15	22.4	2.2	22.1	21.1-23.3	G	33	24.5	3.5	24.0*	22.1-26.3	**0.028**
16	rs932443	AA	22	23.2	2.6	22.6	21.7-24.3	G	26	24.5	3.7	23.9	21.6-26.2	0.179
17	rs2268646	GG	38	24.1	3.5	23.6	21.6-25.8	A	10	23.0	2.3	22.8	21.8-23.8	0.431
18	rs2300614	CC	22	23.7	3.4	22.6	21.6-25.3	T	26	24.0	3.3	23.7	21.7-25.3	0.521
19	rs2268641	GG	22	24.0	3.4	22.6	21.8-26.2	A	25	23.8	3.3	23.7	21.2-25.1	1.000
20	rs2268640	TT	27	23.6	3.1	22.8	21.7-24.7	C	21	24.3	3.5	23.7	21.5-25.3	0.377
21	rs2268639	AA	22	24.0	3.5	22.9	21.6-26.2	T	25	23.8	3.3	23.2	21.5-25.1	0.915
22	rs2206942	GG	18	23.3	2.8	22.3	21.6-24.3	A	30	24.2	3.6	23.7	21.7-25.3	0.277
23	rs2894420	GG	11	24.4	3.0	23.7	22.5-25.7	A	36	23.7	3.4	22.8	21.5-25.2	0.393
26	rs4714211	AA	13	23.4	3.8	22.2	20.6-25.1	G	34	24.1	3.1	23.6	22.1-25.3	0.274
27	rs10305525	CC	39	23.7	3.2	22.9	21.5-25.2	A	9	24.7	3.8	23.2	22.6-26.0	0.420
28	rs9296291	TT	28	23.5	3.1	22.6	21.6-24.6	C	20	24.5	3.5	24.2	22.1-25.9	0.202
29	rs9968886	GG	36	24.0	3.6	23.2	21.4-25.5	A	12	23.5	2.1	23.1	22.5-24.3	0.886
30	rs2143733	TT	14	23.2	3.0	22.0	21.6-24.3	G	34	24.2	3.4	23.6	22.1-25.3	0.229
31	rs9296292	TT	24	23.7	3.3	22.6	21.6-25.3	C	24	24.1	3.4	23.4	22.0-25.3	0.496

Reported *P*-values are for median data. *Significantly faster emptying rate compared to other phenotype ($P < 0.05$).

Table 13. Body fat percentage according to genotype.

		Homozygous Minor Allele					Heterozygous					Homozygous Major Allele					
	SNP ID	**Genotype**	**Mean**	**SD**	**Median**	**Quartiles**	**Genotype**	**Mean**	**SD**	**Median**	**Quartiles**	**Genotype**	**Mean**	**SD**	**Median**	**Quartiles**	***P*-value**
1	rs7738586	---	--	--	--	--	CA	18.1	7.4	16.2	12.3-22.2	CC	19.2	5.9	18.4	15.0-22.1	0.517
2	rs9380825	AA	18.9	8.7	15.5	12.1-26.8	AG	19.2	6.1	18.7	14.1-22.2	GG	18.6	5.9	17.5	15.3-21.5	0.912
4	rs926674	TT	17.6	3.9	15.4	15.3-18.8	TC	22.7	5.9	20.5	18.1-26.4	CC	18.5	6.3	18.0	13.8-22.2	0.282
5	rs2268657	GG	17.7	7.1	14.9	14.2-18.7	AG	19.4	6.2	18.6	13.8-22.3	AA	19.4	6.3	17.5	15.3-22.7	0.630
6	rs13202369	GG	15.7	2.6	15.7	14.7-16.6	AG	20.3	6.0	19.6	16.9-23.2	AA	18.1	6.4	15.5	13.8-22.1	0.295
7	rs3799707	TT	21.1	8.7	21.6	16.9-25.6	GT	19.3	6.3	18.7	14.6-22.3	GG	18.5	6.1	17.7	14.0-21.8	0.811
8	rs10305432	CC	20.8	9.6	15.4	15.3-23.7	CT	18.9	6.2	18.6	13.8-22.7	TT	18.8	6.1	18.1	14.3-22.1	0.953
9	rs9283907	AA	10.0	-	10.0	10.0-10.0	AG	20.9	4.1	21.9	17.5-22.1	GG	18.8	6.5	18.0	13.8-22.1	0.102
10	rs742764	CC	20.8	7.6	20.6	15.4-24.3	TC	18.5	5.9	17.5	13.7-22.1	TT	19.2	6.2	18.7	14.6-22.1	0.670
11	rs2254336	TT	20.8	8.1	21.6	15.2-27.8	TA	18.1	4.9	17.9	14.6-19.5	AA	19.3	6.9	18.7	13.4-23.6	0.767
12	rs910163	CC	20.5	9.0	15.4	15.3-23.2	TC	20.0	5.4	19.3	17.6-22.9	TT	18.2	6.6	16.9	13.3-21.3	0.407
14	rs6923761	AA	17.5	5.7	14.9	14.2-20.6	GA	18.7	6.0	18.0	13.8-22.0	GG	20.4	7.0	19.6	15.4-24.3	0.557
15	rs7766663	GG	21.9	7.1	21.9	16.5-26.9	GT	18.6	5.7	17.9	14.0-22.0	TT	17.7	6.1	16.1	13.0-20.1	0.245
16	rs932443	GG	24.2	8.3	27.8	15.4-30.9	AG	18.7	5.5	18.6	13.8-22.1	AA	18.1	6.1	17.2	13.9-21.7	0.265
17	rs2268646	--	--	--	--	--	AG	17.9	6.0	18.1	13.1-19.2	GG	19.3	6.3	18.3	14.3-23.0	0.603
18	rs2300614	TT	21.0	6.4	21.6	15.4-22.1	CT	19.1	6.3	18.6	13.8-22.1	CC	18.4	6.3	17.1	13.9-22.1	0.658
19	rs2268641	AA	19.8	7.5	16.5	15.4-20.9	AG	19.5	6.2	18.9	13.8-22.1	GG	18.5	6.4	17.8	13.9-22.3	0.794
20	rs2268640	CC	20.5	9.0	15.4	15.3-23.2	TC	20.5	6.2	20.8	16.1-24.0	TT	17.8	5.9	17.5	13.3-20.1	0.274
21	rs2268639	TT	20.5	9.0	15.4	15.3-23.2	TA	19.1	6.4	18.9	13.8-22.1	AA	18.8	6.1	17.8	14.0-22.3	0.941
22	rs2206942	AA	20.9	6.0	19.9	16.3-23.5	AG	19.8	6.6	18.8	15.1-23.3	GG	17.3	5.6	15.9	13.8-20.9	0.247
23	rs2894420	AA	17.8	5.3	17.5	14.5-21.6	AG	18.8	6.8	17.9	13.8-22.7	GG	20.7	5.8	20.9	17.2-23.2	0.406
26	rs4714211	GG	18.2	6.7	15.4	14.5-20.9	AG	19.3	6.1	18.6	14.0-23.8	AA	17.9	5.5	18.0	14.5-19.3	0.827
27	rs10305525	--	--	--	--	--	CA	19.6	7.0	18.9	18.0-20.9	CC	18.8	6.1	17.5	14.0-22.3	0.653
28	rs9296291	CC	20.5	9.0	15.4	15.3-23.2	TC	20.9	6.2	21.9	17.9-24.3	TT	17.7	5.8	17.2	13.5-19.7	0.163
29	rs9968886	--	--	--	--		GA	18.3	6.4	18.1	14.9-19.7	GG	19.2	6.2	18.3	14.1-22.7	0.703
30	rs2143733	GG	18.7	5.9	16.1	15.3-19.9	GT	19.8	6.8	18.8	14.6-25.6	TT	17.5	5.1	15.9	13.9-20.9	0.601
31	rs9296292	CC	18.8	8.1	15.3	14.9-19.3	CT	20.0	6.3	20.3	15.2-23.5	TT	18.2	6.0	17.5	13.5-19.9	0.497

Reported *P*-values are for median data.

Table 14. Body fat percentage according to phenotype.

		Homozygote Major Allele						Heterozygote/homozygote minor allele						
	SNP ID	**Phenotype**	**n**	**Mean**	**SD**	**Median**	**Quartiles**	**Phenotype**	**n**	**Mean**	**SD**	**Median**	**Quartiles**	***P*-value**
1	rs7738586	CC	38	19.2	5.9	18.4	15.0-22.1	A	10	18.1	7.4	16.2	12.3-22.2	0.517
2	rs9380825	GG	15	18.6	5.9	17.5	15.3-21.5	A	33	19.2	6.4	18.7	13.8-22.5	0.876
4	rs926674	CC	39	18.5	6.3	18.0	13.8-22.2	T	9	21.0	5.7	18.8	17.5-22.1	0.224
5	rs2268657	AA	11	19.4	6.3	17.5	15.3-22.7	G	36	19.0	6.3	18.7	13.8-22.2	0.841
6	rs13202369	AA	25	18.1	6.4	15.5	13.8-22.1	G	23	19.9	5.9	18.9	16.5-22.9	0.265
7	rs3799707	GG	26	18.5	6.1	17.7	14.0-21.8	T	22	19.5	6.4	18.7	14.4-22.4	0.576
8	rs10305432	TT	26	18.8	6.1	18.1	14.3-22.1	C	22	19.2	6.5	18.3	14.2-22.9	0.868
9	rs9283907	GG	38	18.8	6.5	18.0	13.8-22.1	A	10	19.8	5.2	21.8	17.1-22.1	0.446
10	rs742764	TT	14	19.2	6.2	18.7	14.6-22.1	C	33	19.0	6.3	17.9	13.8-22.1	0.871
11	rs2254336	AA	16	19.3	6.9	18.7	13.4-23.6	T	32	18.9	5.9	18.0	14.7-22.1	0.948
12	rs910163	TT	27	18.2	6.6	16.9	13.3-21.3	C	21	20.0	5.7	18.9	15.5-23.2	0.183
14	rs6923761	GG	13	20.4	7.0	19.6	15.4-24.3	A	35	18.5	5.9	17.9	13.8-22.0	0.318
15	rs7766663	TT	15	17.7	6.1	16.1	13.0-20.1	G	33	19.6	6.3	18.6	15.2-23.2	0.322
16	rs932443	AA	22	18.1	6.1	17.2	13.9-21.7	G	26	19.8	6.3	18.7	15.3-23.8	0.362
17	rs2268646	GG	38	19.3	6.3	18.3	14.3-23.0	A	10	17.9	6.0	18.1	13.1-19.2	0.603
18	rs2300614	CC	22	18.4	6.3	17.1	13.9-22.1	T	26	19.5	6.2	18.7	15.3-22.1	0.548
19	rs2268641	GG	22	18.5	6.4	17.8	13.9-22.3	A	25	19.6	6.2	18.8	15.2-22.1	0.502
20	rs2268640	TT	27	17.8	5.9	17.5	13.3-20.1	C	21	20.5	6.4	19.6	15.4-24.3	0.112
21	rs2268639	AA	22	18.8	6.1	17.8	14.0-22.3	T	25	19.3	6.5	18.8	14.2-22.1	0.773
22	rs2206942	GG	18	17.3	5.6	15.9	13.8-20.9	A	30	20.0	6.4	18.9	15.4-23.8	0.115
23	rs2894420	GG	11	20.7	5.8	20.9	17.2-23.2	A	36	18.6	6.4	17.7	13.8-22.2	0.187
26	rs4714211	AA	13	17.9	5.5	18.0	14.5-19.3	G	34	19.0	6.2	18.3	13.9-22.9	0.634
27	rs10305525	CC	39	18.8	6.1	17.5	14.0-22.3	A	9	19.6	7.0	18.9	18.0-20.9	0.653
28	rs9296291	TT	28	17.7	5.8	17.2	13.5-19.7	C	20	20.8	6.4	20.8	15.5-24.9	0.061
29	rs9968886	GG	36	19.2	6.2	18.3	14.1-22.7	A	12	18.3	6.4	18.1	14.9-19.7	0.703
30	rs2143733	TT	14	17.5	5.1	15.9	13.9-20.9	G	34	19.6	6.6	18.8	15.3-23.8	0.335
31	rs9296292	TT	24	18.2	6.0	17.5	13.5-19.9	C	24	19.8	6.5	19.3	15.0-23.5	0.312

Reported *P*-values are for median data.

6.4. DISCUSSION

The results of this study showed several Tag SNPs of the GLP-1R gene are significantly associated with gastric emptying rate of a glucose solution in Caucasian men. Two neighbouring SNPs 10 and 11, rs742764 and rs2254336, were found to be significantly associated with gastric emptying rate by genotype. In addition SNP 5 rs2268657, SNP 9 rs9283907 and SNP 15 rs7766663 tended to significance. Furthermore, three SNPs, 5, 9 and 11, rs2268657, rs9283907 and rs2254336, respectively, were found to be significantly associated with gastric emptying rate by phenotype. SNP 15 rs7766663 also tended to significance. Thus, significant associations between genetic variation and gastric emptying rate were found for four Tag SNPs; 5, 9, 10 and 11 by one or more measures of genetic association. The former SNP is situated in intron 1 and the latter three situated in intron 3. SNP 11 had a significant association by both genotype and phenotype, SNP 10 had a significant association by genotype, and SNPs 9 and 5 had a tendency of genotype association and a significant association by phenotype. A tendency of significance by genotype and phenotype was also found for SNP 15 which is situated in intron 5.

The aforementioned Tag SNPs identified to be associated with gastric emptying rate signify a region(s) where a causative variant is most likely to reside (Xia & Grant, 2013). As three of the associated SNPs are neighbouring SNPs, this presents a genomic region that warrants further investigation with particularly high interest. Approximately 3000 bp exist between the locations of SNP 9 and 10 and similarly between SNP 10 and 11. Spanning the whole 6132 bp 'hot spot' region between SNP 9 and 11, a total of 129 SNPs have been sequenced to date. No known functional SNPs within the GLP-1R gene have currently been identified within this particular region or within introns 1, 3 or 5. However, it is widely known that SNPs in regulatory elements residing within intronic regions can alter silencing, enhancer, or splicing events. Further work by *in silico* analysis or multi-array analysis of all 129 known SNPs within this region to narrow down on the precise SNP or SNPs responsible for the observed differences in gastric emptying rate should therefore be conducted. The genetic variants surrounding SNP 5 in intron 1 should also be further investigated as SNPs within intron 1 of several genes have been shown to influence gene transcription events.

Variants in the proximity of SNP 15 also provide a directive area of further research in gastric emptying regulation as it tended toward significance but was also significantly associated with BMI by phenotype. This association may signal a link between gastric emptying rate and BMI but further participants are required to confirm

these concurrent associations. Indeed, further potential 'links' or associations may also be identified with a much larger sample size which will increase the power of future studies. The limitation in sample size and the fact that all participants were healthy and predominantly classed as normal weight according to BMI and had normal body fat percentage, with a few exceptions, may explain the lack of correlation between gastric emptying rate and BMI and body fat percentage seen in this study.

The two additional missense SNPs in close proximity to the locus of the variant seen in the mice model by Kumar *et al*., (2008) did not show any variants in the sample population of this present study. It may be that the variant is rare or non-existent in the Caucasian population. The minor allele frequency (MAF) for these two missense SNPs are unknown. The other additional missense SNP selected for its previous association with insulin secretion (Sathananthan *et al*., 2010) also showed no variants in this participant group. This was mostly likely due to its small MAF of 0.0646 indicating the SNP is somewhat rare. Tag SNPs 6 rs13202369 (intron 1) and 14 rs6923761 (exon 5) have previously been reported to alter insulin secretion responses to intravenous GLP-1 (Sathananthan *et al*., 2010; Vella *et al*., 2009). These were not found to be associated with gastric emptying rate in this study, however.

In conclusion, the results of this targeted gene study to investigate the potential influence of GLP-1R genetic variation on gastric emptying rate in humans revealed several Tag SNPs to be associated with gastric emptying rate of a glucose solution in healthy Caucasian men. This suggests that genetic variation within the GLP-1R gene may influence gastric emptying rate in humans. Further work should be undertaken to identify the precise SNP or SNPs responsible and functional analysis conducted. Furthermore, this association study should be repeated with a larger population sample to independently confirm the detected associations between GLP-1R genetic variation and gastric emptying rate.

7. GENERAL DISCUSSION AND CONCLUSIONS

The growing prevalence of overweight and obesity is a major health concern worldwide. In an attempt to understand and tackle the rising problem of obesity, much research has been conducted on subjective feelings of appetite, gut hormone secretion and food intake, and how interventions such as physical activity and exercise may affect these. The gastrointestinal system, in particular the role of gastric emptying, in appetite and satiety is often overlooked. Greater understanding of the physiology of gastric emptying and its interactions with nutrients could help in the development of dietary interventions that can affect appetite and modulate energy intake. The aims of this thesis were to determine some of the hormonal and genetic influences on gastric emptying characteristics and to investigate the intrinsic link between the regulation of gastric emptying and the regulation of appetite with particular focus on dietary carbohydrates and fructose ingestion. The results of the studies presented in this thesis centre around four main themes that are discussed below.

7.1. GASTRIC EMPTYING RATE OF DIFFERENT CARBOHYDRATE SOLUTIONS

The effect of different carbohydrate solutions on gastric emptying rate is an important consideration in the pathophysiology of obesity and metabolic syndrome resulting from fructose and sugary drink overconsumption. Previous investigations comparing the gastric emptying rate of different carbohydrate solutions have shown inconsistent results. Several studies have previously reported a monosaccharide fructose solution to empty faster than an isoenergetic glucose solution (Horowitz *et al*., 1996; Sole & Noakes, 1989; Moran & McHugh, 1981; Elias *et al*., 1968) and a sucrose solution (Elias *et al*., 1968). In turn, glucose has been reported to empty faster than sucrose (Murray *et al*., 1994) though a combined mixture of fructose and glucose has been shown to empty faster than glucose (Jeukendrup & Moseley, 2010). On the other hand, some studies have reported no difference in emptying rate between fructose and glucose (McGlone *et al*., 2008, Shi *et al*., 2000) and no difference between sucrose, combined fructose and glucose and glucose alone (Shi *et al*., 2000).

The study presented in chapter 3 of this thesis was designed to investigate the gastric emptying rate of a number of different commonly ingested carbohydrate solutions; glucose, fructose, sucrose and combined glucose and fructose solution. There appeared to be a faster emptying of sucrose than glucose as reflected by the longer T_{lag} for glucose than sucrose. Another interesting observation is the large inter individual variation in rates of gastric emptying, which may have a genetic basis, as will be discussed below.

In addition, it is clear from the studies reported in chapters 4 and 5 of this thesis that a fructose solution empties from the stomach at a faster rate than a glucose solution. Although these two studies were not designed to directly compare the emptying rate of glucose to fructose, the available data shows the emptying rate of fructose is significantly faster than glucose both before and after increased dietary fructose supplementation. The faster emptying rate of fructose or fructose containing solutions compared to glucose seen throughout this thesis may suggest a mechanistic link between the argued development of fructose associated obesity and metabolic syndrome. Further work to investigate this is required.

The variation and differences in gastric emptying rate between the different types of carbohydrate observed in this thesis cannot be attributed to volume, osmolality or energy content. Carbohydrate solutions in the studies presented in chapters 3, 4 and 5 were of equal volume, energy content, and with the exception of sucrose in chapter 3, equal osmolality. Sucrose with its lower osmolality due to its disaccharide form did not empty the quickest, however. This suggests an interactive sensing mechanism relating to the carbohydrate molecule itself rather than solely osmoreceptors or energy nutrient sensors in the gastrointestinal tract. Differences in transporter activation and absorption may play a role but further work is required to explore this possibility.

7.2. HORMONAL AND METABOLIC RESPONSES TO DIFFERENT CARBOHYDRATE SOLUTIONS

Gastric emptying is regulated by a complex interaction of neuronal and hormonal input. Many of these hormones produced and secreted by the gastrointestinal tract have simultaneous effects on appetite regulation. Previous investigations have shown fructose ingestion stimulates GLP-1 (Kong *et al*., 1999), insulin (Teff *et al*., 2009; Bowen *et al*., 2007; Kong *et al*., 1999) and leptin (Bray, 2010) secretion, and suppresses ghrelin secretion (Teff *et al*., 2004) to a lesser extent than isoenergetic amounts of glucose. The difference in GLP-1 and ghrelin responses have not consistently been shown, however. No difference in the responses of these two gut hormones was reported by Bowen *et al*. (2007). Furthermore, a previous investigation has found no difference in plasma insulin, leptin and ghrelin between the similar carbohydrates HFCS and sucrose (Melanson *et al*., 2007). However, these studies have all involved the ingestion of very large and unrealistic amounts of glucose, fructose and other sugars. In addition, the studies that have measured ghrelin have consistently examined total ghrelin and not the active form acylated ghrelin.

Due to its differential handling by the liver compared to glucose, the consumption of large amounts of fructose in the diet is also suggested to contribute to the development of NAFLD. Previous investigations have shown that short to moderate term overfeeding with fructose results in increased fasting and postprandial plasma triglyceride concentrations (Stanhope *et al*., 2011; Ngo Sock *et al*., 2010; Stanhope *et al*., 2009; Teff *et al*., 2009; Stanhope *et al*., 2008; Teff *et al*., 2004; Bantle *et al*., 2000), and decreased lipolysis and metabolism of NEFA (Ngo Sock *et al*., 2010; Le *et al*., 2006; Teff *et al*., 2004) compared with glucose. Previous studies that have investigated the effect of ingestion of a single acute bolus solution of carbohydrate have reported increased blood lactate and NEFA concentrations for mixed glucose and fructose solution compared with glucose alone (Bidwell *et al*., 2010) but inconsistent triglyceride responses. One study showed no difference (Bidwell *et al*., 2010) and another showed fructose-containing solutions resulted in greater triglyceride response compared with glucose alone (Parks *et al*., 2008). Furthermore, studies that have compared the acute effects of HFCS and sucrose have found no differences in postprandial triglyceride or lactate responses (Le *et al*., 2012; Stanhope *et al*., 2008). However, all of these studies have again used untypical amounts of carbohydrate ingestion ranging from 50 g to approximately 135 g (30% of estimated energy requirements). A typical 330 mL serving of some commercially-available soft drinks contain approximately 35 g to 37 g of sugar. The studies presented in chapters 3 and 5 of this thesis were designed to investigate gastrointestinal hormone responses and hepatic metabolism of more typical amounts of carbohydrate ingestion. These studies were also designed to measure gastrointestinal hormones and metabolic responses concurrently with gastric emptying rate in order to give light on the mechanisms and effects of the regulation of gastric emptying rate to the ingestion of different carbohydrates. From the data presented in these chapters, it was observed that the ingestion of a single acute bolus solution of fructose in typical amounts induce significantly lower GIP and reduced insulin responses compared to all other carbohydrate solutions. Furthermore, for all carbohydrate solutions the responses of GIP mirrored that of insulin more closely than the responses of GLP-1 with insulin, suggesting a predominant role of the incretin GIP in glucose stimulated insulin release rather than the incretin GLP-1. No differences in overall GLP-1 secretion or ghrelin suppression were seen but the pattern of GLP-1 response differed with peak GLP-1 concentration occurring later during fructose ingestion compared to other carbohydrate solutions. This suggests the faster emptying rate of fructose may be attributable to delayed GLP-1 secretion. However, further work is required.

The ingestion of a single acute bolus of fructose or glucose following increased dietary fructose consumption of 120 g/d for a period of three consecutive days lead to some subtle changes in the profile of postprandial gastrointestinal hormone responses. During fructose and glucose ingestion, ghrelin concentration was slightly higher at baseline and within the first 30 min for each respective supplement trial compared with control. GIP and GLP-1 responses were also slightly elevated during glucose ingestion but unchanged and slightly suppressed for fructose ingestion. These slight alterations in hormones known to influence gastric emptying rate may explain the changes in gastric emptying rate observed.

It can also be seen from the data presented in chapters 3 and 5 that the acute ingestion of a typical amount of fructose alone or in combination with free or bound glucose results in significant production of lactate and is significantly greater than when glucose is ingested alone. It does not result in significant increases in one-hour postprandial triglyceride, nor differences between carbohydrates in NEFA or D-3-hydroxybutyrate concentration, however, suggesting that acute fructose ingestion in typical amounts does not cause an increase in *de novo* lipogenesis and results in similar reductions in lipolysis and NEFA metabolism to other carbohydrate solutions. On the other hand, the data indicates that increased dietary fructose consumption of 120 g/d for even a period of three consecutive days may lead to deleterious increases in triglyceride concentration but to levels below that of dyslipidaemia.

7.3. EFFECT OF DIETARY SUPPLEMENTATION OF FRUCTOSE ON GASTRIC EMPTYING RATE

Increased dietary consumption of fructose is argued to play a role in the pathogenesis of obesity and metabolic syndrome by altering feeding patterns and promoting weight gain. As gastric distension stimulates satiation and satiety (Geliebter *et al*., 1988), the effect of increased fructose ingestion on gastric emptying rate is an important area to study. Previous investigations have indicated that gastric emptying in humans may be influenced by patterns of previous dietary intake. In addition, there is evidence to suggest that these adaptive changes are macronutrient specific and rapid, with adaptation occurring in a few days. For example, a high fat diet for 14 d has been shown to accelerate gastric emptying of a high fat meal (Cunningham *et al*., 1991) but not a high carbohydrate meal (Castiglione *et al*., 2002). This has also been reported following only 3 d of a high fat diet (Clegg *et al*., 2011). Increased ingestion of glucose by 400 g per day for 3 d has also been shown to accelerate gastric emptying of a hyperosmotic glucose solution but not a protein

solution (Cunningham *et al*., 1991). The effects of glucose supplementation have not been reported to be monosaccharide specific however as the emptying of a hyperosmotic fructose solution was also equally accelerated (Horowitz *et al*., 1996).

The study reported in chapter 4 of thesis was designed to investigate the effect of dietary supplementation of fructose for 3 d on gastric emptying rate of fructose and glucose. This study was then repeated with the addition of acquiring gut hormone response data and is presented in chapter 5 of this thesis. Data from these two studies indicate that short-term supplementation of the diet with 120 g fructose per day for 3 d results in significantly accelerated gastric emptying rate of a 6% fructose solution but not a 6% glucose solution. These data therefore show a monosaccharide specific adaptation of the gut to increased fructose in the diet. The data also demonstrates an adaptation of gastric emptying rate to a much smaller amount of additional carbohydrate consumption compared to amounts utilised in previous studies. Although the supplementation dose of fructose (120 g/d) ingested in the studies presented in this thesis is four times the amount of this typical single serving, data shows that it is not an unrealistic amount. Estimated daily mean, 90th and 95th percentile fructose intakes from NHANES data are reported respectively as 63 g, 103 g and 118 g for males aged 23-50 y and 75 g, 117 g and 134 g for males aged 19-22 y (Marriott *et al*., 2009). The effect of a longer period of high fructose intake on gastric emptying rate of different carbohydrates is unknown and whether adaptations revert back within the same time frame should be investigated further.

7.4. INFLUENCE OF GENETICS ON GASTRIC EMPTYING RATE

A role of genetics in the susceptibility of excess weight accumulation and obesity has been given much attention and has been established. Research on the influence of genetic variation on gastric emptying rate has been scarce, however. Previous investigations have reported common genetic variants in the MC4R gene (Acosta *et al*., 2014) and the CCK gene (Cremonini *et al*., 2005) to be associated with gastric emptying rate in humans. One study conversely found no association of genetic variants of the CCK or CCK-1 receptor genes on gastric emptying rate (Jones *et al*., 2010).

The studies in chapters 3, 4 and 5 of this thesis showed large variation in gastric emptying rate, particularly for a glucose solution. As GLP-1 is known to be potently released in response to glucose ingestion and is also known to slow gastric emptying, the GLP-1R was selected as a candidate gene for investigation. In addition, a previous investigation had reported an influence of GLP-1R genetic variation on gastric emptying

rate in mice (Kumar *et al*., 2008). The study in Chapter 6 of this thesis was therefore designed to investigate the influence of genetic variation in the GLP-1R gene on gastric emptying rate in humans.

Several Tag SNPs within the GLP-1R gene were significantly associated with gastric emptying rate of a glucose solution in Caucasian men. The Tag SNPs identified signify a region(s) where a causative variant is most likely to reside (Xia & Grant, 2013) and where further work such as *in silico* analysis and functional analysis is required to establish the precise SNP or SNPs responsible for the observed differences in gastric emptying rate. In addition, several of these SNPs were neighbouring SNPs, indicating a 'hot spot' genomic region that warrants further investigation with particular high interest. Furthermore, this association study should be repeated with a larger population sample to independently confirm the detected associations between GLP-1R genetic variation and gastric emptying rate.

7.5. LIMITATIONS OF METHODOLOGY

A limitation of the studies in this thesis is the small sample size. In particular, the study reported in chapter 3 of this thesis included data from seven participants only. As discussed, the combined interaction of a relatively small sample size, the large inter-individual variation for gastric emptying rate of glucose containing solutions and the number of conditions for comparison were likely responsible for the lack of statistical difference of gastric emptying rate between conditions. In addition, the genetics study reported in chapter 6 of this thesis had a sample size of forty-eight participants. Although ideal sample sizes for gene candidate studies enter into triple and quadruple figures (Zondervan & Cardon, 2007), the results of the study reported in this thesis nevertheless indicated several significant associations between several Tag SNPs and gastric emptying parameters. The study in chapter 6 of this thesis would therefore require replication with a much larger cohort to confirm the associations.

All studies were conducted with healthy individuals with a normal BMI and only a handful had a BMI indicative for being just above 'obese'. Thus, the results of these studies may not be applicable to the clinical obese or morbidly obese population. Furthermore, whether differences in gastric emptying characteristics, gastrointestinal hormone secretion and hepatic fructose metabolism, as well as genetic associations exist between normal weight and obese populations remain to be elucidated.

Another methodological limitation is that measurements of appetite were collected using VAS scales. Whilst VAS scales are a valid, reliable and reproducible

indicator and predictor of appetite and feeding behaviour (Flint *et al*., 2000; Stubbs, Hughes, Johnstone, Rowley, Reid, Elia *et al*., 2000), they are often a poor proxy of actual energy intake (Stubbs *et al*. 2000). Measurement of subsequent energy intake, perhaps via an *ad libitum* meal, would provide more translational and nutritional information on the effects of various carbohydrates solutions and fructose supplementation.

A further methodological limitation is that the control trials in the supplementation studies described in chapters 4 and 5 did not involve the ingestion of placebo sweetened supplement drinks. Although this would have been unlikely to consciously or subconsciously affect gastric emptying rate or hormone responses of the test solutions, it may potentially affect adherence to ingestion of the same diet during the dietary maintenance periods. In addition, with respect to the dietary intake during the maintenance period, the diet was not prescribed nor provided and it was entrusted to the participants that they had ingested the exact same foods and diet prior to each experimental trial as they were instructed to.

Gastric emptying was assessed in this thesis using the ^{13}C breath test. Although this method has been validated and correlated strongly with the gold standard method scintigraphy and gastric aspiration (Braden *et al*., 2004; van Nieuwenhoven *et al*., 1999; Pfaffenbach *et al*., 1996; Braden *et al*., 1995; Ghoos *et al*., 1993) this method is an indirect assessment of gastric emptying rate. However, this simple, non-radioactive and non-invasive technique allows commensurate application for the assessment of gastric emptying of solid food in future studies to follow on from the present studies on liquid solutions conducted within this thesis.

7.6. CONCLUSIONS

The experimental studies described within this thesis have extended knowledge on the effects of carbohydrate ingestion on gastric emptying rate and gut hormone responses and the influence of genetics on gastric emptying rate in humans. The main conclusions that can be drawn from this work are:

1) Six percent carbohydrate solutions of equal energy content and volume empty from the stomach at varying rates with fructose alone emptying faster compared to glucose alone. Furthermore, a sensing mechanism independent of osmolality may be responsible for this observation.

2) Ingestion of different carbohydrate solutions induce different gastrointestinal/appetite hormone responses. Ingestion of a 6% fructose solution results in markedly reduced and virtually absent GIP and insulin secretion responses compared with ingestion of a 6% glucose solution. Overall acylated ghrelin and active GLP-1 responses do not differ between carbohydrates, however.

3) Ingestion of an acute bolus of fructose or fructose-containing solution at typical amounts results in significant production of lactate compared to the ingestion of an isoenergetic solution of glucose. It does not cause immediate increases in serum triglyceride, nor differences between carbohydrates in NEFA or D-3-hydroxybutyrate concentration, however, suggesting that ingestion of fructose in typical amounts does not cause an increase in *de novo* lipogenesis and results in similar reductions in lipolysis and NEFA metabolism to other carbohydrate solutions. Increased dietary fructose consumption of 120 g/d for even a period of three consecutive days may, on the other hand, have deleterious effects on triglyceride concentration.

4) Gastric emptying is a highly adaptable process that is influenced by previous dietary intake. A short-term increase in fructose ingestion for only three consecutive days accelerates gastric emptying rate of a fructose solution but not a glucose solution. This monosaccharide adaptation may be unique to fructose. The mechanism of adaptation requires further investigation but may be due to subtle differences in gastrointestinal hormone response changes.

5) Gastric emptying rate of a glucose solution varies prominently between individuals and genetic variation of the GLP-1R may explain some of this variation in gastric emptying rate.

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APPENDICES

APPENDIX 1: ETHICAL APPROVAL LETTERS

Study 1 (Chapter 3)

FACULTY OF SCIENCE AND ENGINEERING

Manchester Metropolitan University

MEMORANDUM

TO Dr Gethin Evans

FROM Will Smith

DATE

SUBJECT Faculty Ethics Committee Application

At the Faculty Ethics Committee held on Wednesday, 2nd February, 2011 The Committee considered an application for Ethical Approval from Mr Gethin Evans entitled "The effects of different types of carbohydrate on rate of gastric emptying and perceived hunger"

The application received a favourable opinion from the committee and was approved.

The Committee requires that you report any Adverse Event during this study immediately to the Chair and Committee Secretary. Adverse Events are adverse reactions to any modality, drug or dietary supplement administered to subjects or any trauma resulting from procedures in the protocol of a study.

An Adverse Event may also be accidental loss of data or loss of sample, particularly human tissue. Loss of human tissue or cells must also be reported to the designated individual for the Human Tissue Authority licence (currently Prof Bill Gilmore).

Regards

Will Smith
Student Information Point
All Saints North (John Dalton Building)
http://www.mmu.ac.uk/sas

Study 2 (Chapter 4)

FACULTY OF SCIENCE AND ENGINEERING

Manchester Metropolitan University

M E M O R A N D U M

TO Mo Wah Adora Yau

FROM AnneMarie Walsh

DATE

SUBJECT Faculty Ethics Committee Application (**SE111202)**

At the Faculty Ethics Committee held on Wednesday, 26th October, 2011 the Committee considered an application for Ethical Approval from Mo Wah Adora Yau **(SE111202)** entitled "The effect of short term dietary supplementation with fructose on gastric emptying of glucose and fructose"

The Committee requested clarification on the following areas

- Data Storage
- Potential Risks to Participants
- Advice given to participants whom fast
- What questions were contained in the Subjective Questionnaire

The applicant submitted a protocol amendment.

I am pleased to announce that your resubmission has addressed the points raised satisfactorily and your application has received favourable opinion by the Chair on behalf of the committee.

The Committee requires that you report any Adverse Event during this study immediately to the Chair and Committee Secretary. Adverse Events are adverse reactions to any modality, drug or dietary supplement administered to subjects or any trauma resulting from procedures in the protocol of a study.

An Adverse Event may also be accidental loss of data or loss of sample, particularly human tissue. Loss of human tissue or cells must also be reported to the designated individual for the Human Tissue Authority licence (currently Prof Bill Gilmore).

Regards

AnneMarie Walsh
Research Degrees Group Officer
All Saints North

Study 3 (Chapter 5)

FACULTY OF SCIENCE AND ENGINEERING

Manchester Metropolitan University

M E M O R A N D U M

TO Mo Wah Adora Yau/Gethin Evans

FROM AnneMarie Walsh

DATE

SUBJECT Faculty Ethics Committee Application (**SE111228)**

At the Faculty Ethics Committee held on Wednesday, 26th September 2012 the Committee considered an application for Ethical Approval from Mo Wah Adora Yau **(SE111228)** entitled "The effect of short term dietary supplementation with fructose on Gastric Emptying of Glucose and Fructose, and associated gut hormone responses"

The Committee requested clarification on the following areas

- On page 3 of the ethical approval form the authors state that "volunteers who have not participated in any previous studies in our laboratory involving fructose ingestion will be asked to consume a 600ml solution containing 36g of fructose to ensure no unknown intolerance to fructose is experienced prior to further participation". In response, one must ask if the volunteers turn out to be intolerant to fructose, would this not cause them some discomfort or harm? Fructose intolerance should be included in the Exclusion Criteria.
- The language of the Volunteer Information Sheet could be less technical for the layperson who may participate in the study. For example the following terms could be explained or defined in a clearer fashion: what is "metabolic syndrome"? Where/what is the "antecubital vein"? What is "13C-acetate" other than a "commonly used and safe substance"?
- The protocol for making the data anonymous should be explained on the Volunteer Information Sheet.

It was also noted that Risk Assessments should be submitted with the application

The applicant submitted a protocol amendment.

I am pleased to announce that your resubmission has addressed the points raised satisfactorily and your application has received favourable opinion by the Chair on behalf of the committee.

The Committee requires that you report any Adverse Event during this study immediately to the Chair and Committee Secretary. Adverse Events are adverse reactions to any modality, drug or dietary supplement administered to subjects or any trauma resulting from procedures in the protocol of a study.

An Adverse Event may also be accidental loss of data or loss of sample, particularly human tissue. Loss of human tissue or cells must also be reported to the designated individual for the Human Tissue Authority licence (currently Prof Bill Gilmore).

Regards

AnneMarie Walsh
Research Degrees Group Officer
All Saints North

Study 4 (Chapter 6)

FACULTY OF SCIENCE AND ENGINEERING

Manchester Metropolitan University

M E M O R A N D U M

TO Dr Gethin Evans

FROM Will Smith

DATE

SUBJECT Faculty Ethics Committee Application

At the Faculty Ethics Committee held on Wednesday, 6th April, 2011 the Committee considered an application for Ethical Approval from Dr. Gethin Evans titled "The influence of glucagons like peptide-1(GLP-1) receptor genetic variation on gastric emptying characteristics"

Concerns were raised regarding the application and the candidate is to be asked by the Committee Secretary to clarify the following points upon resubmission for Chairs Action.

- The applicant should specify the reason why only Caucasian Males are to be used in the Research.

I am pleased to announce that you recent resubmission has addressed these points satisfactorily and your application has received a favourable opinion from the committee and has been approved.

The Committee requires that you report any Adverse Event during this study immediately to the Chair and Committee Secretary. Adverse Events are adverse reactions to any modality, drug or dietary supplement administered to subjects or any trauma resulting from procedures in the protocol of a study.

An Adverse Event may also be accidental loss of data or loss of sample, particularly human tissue. Loss of human tissue or cells must also be reported to the designated individual for the Human Tissue Authority licence (currently Prof Bill Gilmore).

Regards

Will Smith
Student Information Point
All Saints North (John Dalton Building)
http://www.mmu.ac.uk/sas

APPENDIX 2: STABILITY OF C13 BREATH SAMPLES STORED OVER TIME AND EFFECT ON THE RELIABILITY OF GASTRIC EMPTYING RESULTS

During the study reported in chapter 3 of this thesis, an unforeseen technical problem with the computer associated with the IRIS analyser resulted. Analysis of many collected breath samples were thus delayed by approximately six weeks and were stored for much longer than anticipated. Previously obtained samples were analysed within two weeks of collection. Consequent analysis of the samples that had been stored for an extended period of time showed that the concentration levels of CO_2 had dramatically decreased and in many cases were below 0.5%. No documented guidelines or published studies are available with regards to the length of sample storage time and the reliability of gastric emptying results. A small study was therefore conducted to investigate the effect of sample storage time length on CO_2 concentration reduction and the reliability of gastric emptying results.

Methods

Five healthy volunteers consumed 600 mL of a 6% sucrose solution containing 100 mg sodium acetate within 2 min. Breath samples were collected at baseline (0 min) and every 10 min following drink ingestion for 1 h. It was ensured that the volume of breath sample collected in each foil bag was sufficient for at least two analyses. All samples were analysed in the afternoon of the day of collection and a subsequent analyses was repeated on each set of samples following either 4 d, 1 week, 2 weeks, 4 weeks or 6 weeks of storage.

Results

All five sets of samples were successfully analysed twice. Results for gastric emptying $T_{½}$ are shown in Figure A1 and results for gastric emptying T_{lag} shown in Figure A2. Results were either no different or minimal. The largest differences between analyses were seen for the set of samples re-analysed at 1-week with differences in 6 min and 4 min for $T_{½}$ and T_{lag}, respectively. This was likely due to the originally higher values for gastric emptying rate indicating very delayed emptying. Over 6 weeks, the CO_2% of samples dropped to an average of 0.95% and the average decrease in samples was 3.71%. A curvilinear relationship between the decrease in CO_2% and number of days was established and can be seen in Figure A3. Significant strong correlations between analysis

one and two results were seen for both $T_{½}$ (r = 0.999; $P < 0.001$) and T_{lag} (r = 0.999; $P < 0.001$) (Figure A4).

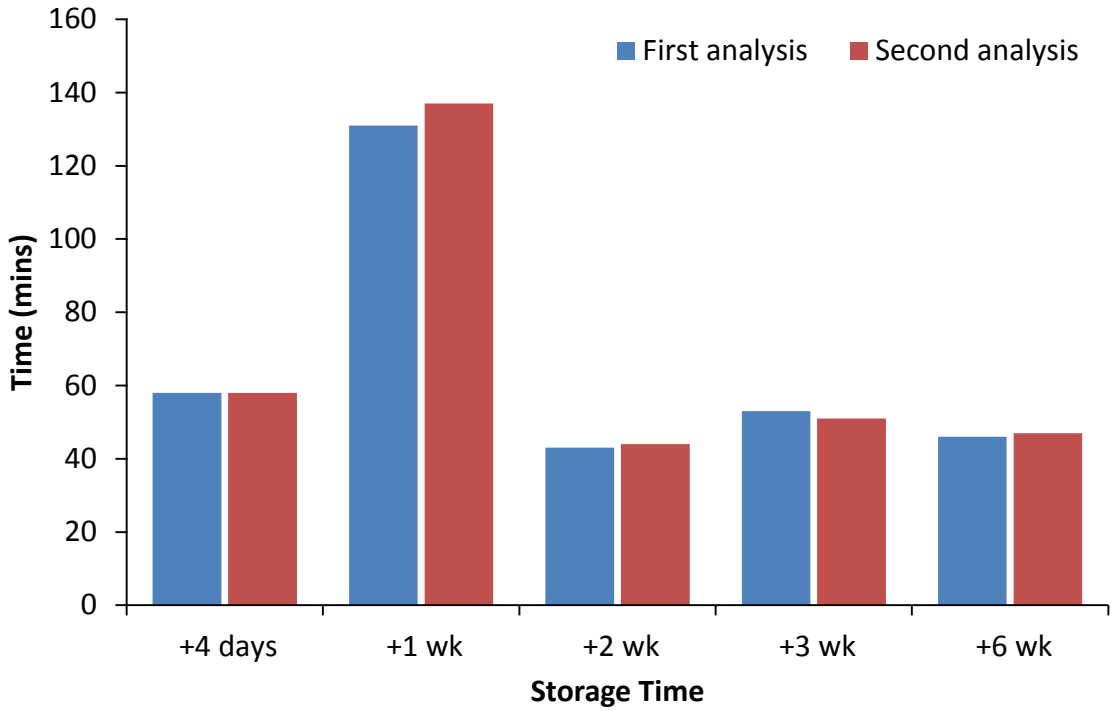

Figure A1. Gastric emptying $T_{½}$ results for each set of samples analysed on the day of sample collection and re-analysed following different lengths of storage time

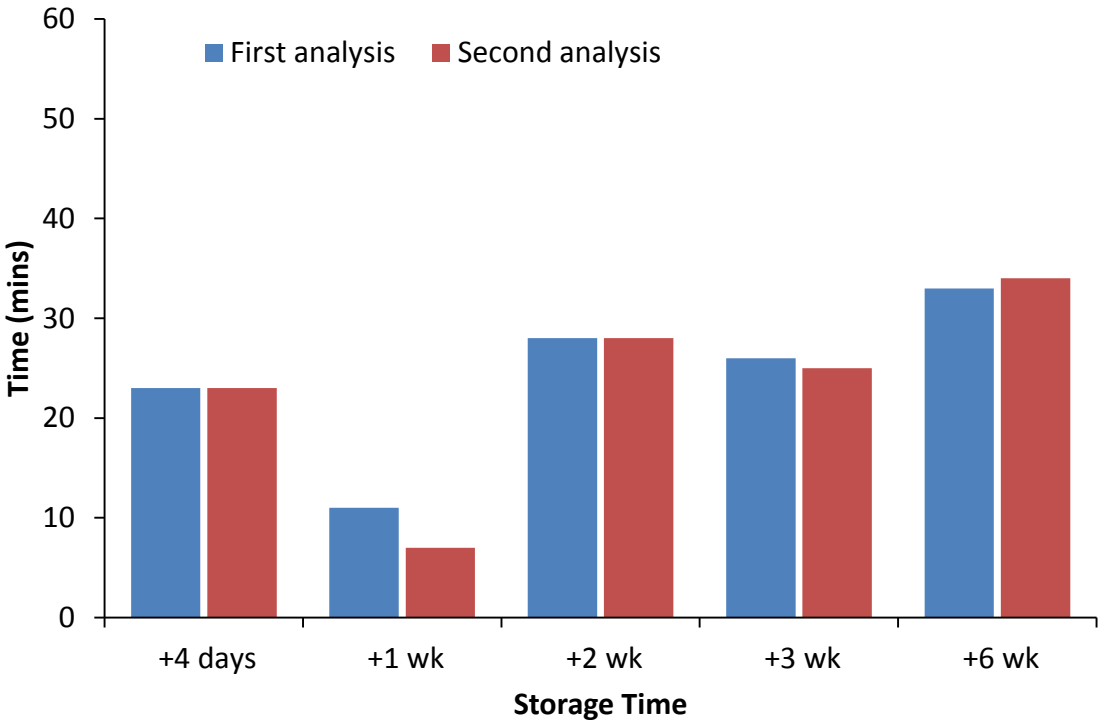

Figure A2. Gastric emptying T_{lag} results for each set of samples analysed on the day of sample collection and re-analysed following different lengths of storage time.

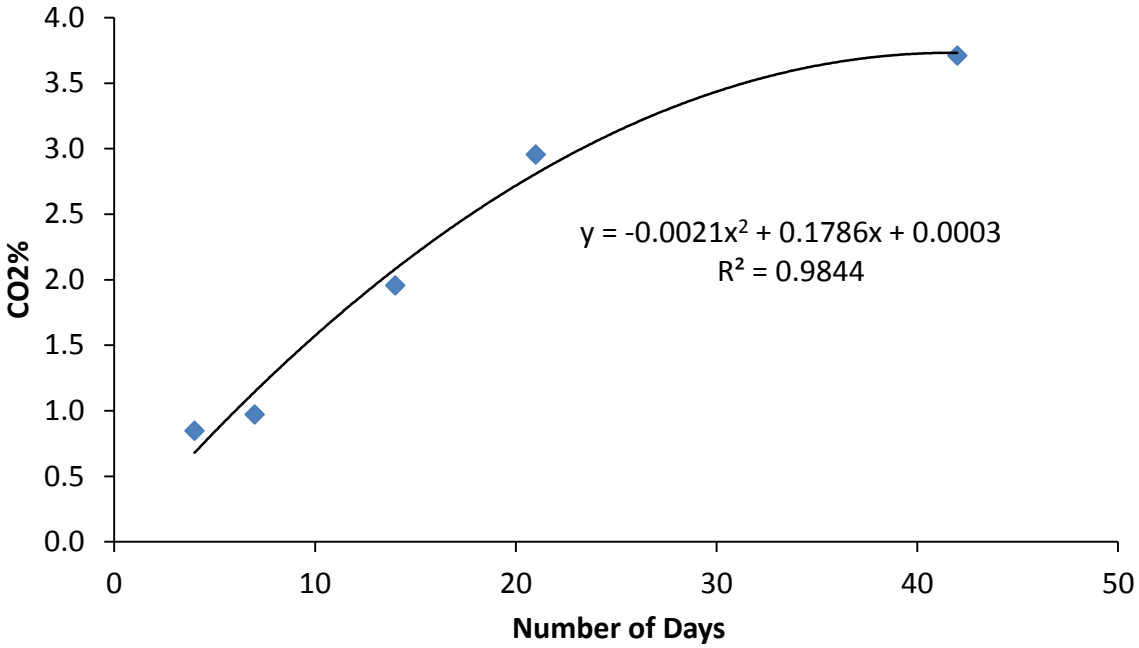

Figure A3. Average decrease in $CO_{2\%}$ following different lengths of storage time over 6 weeks

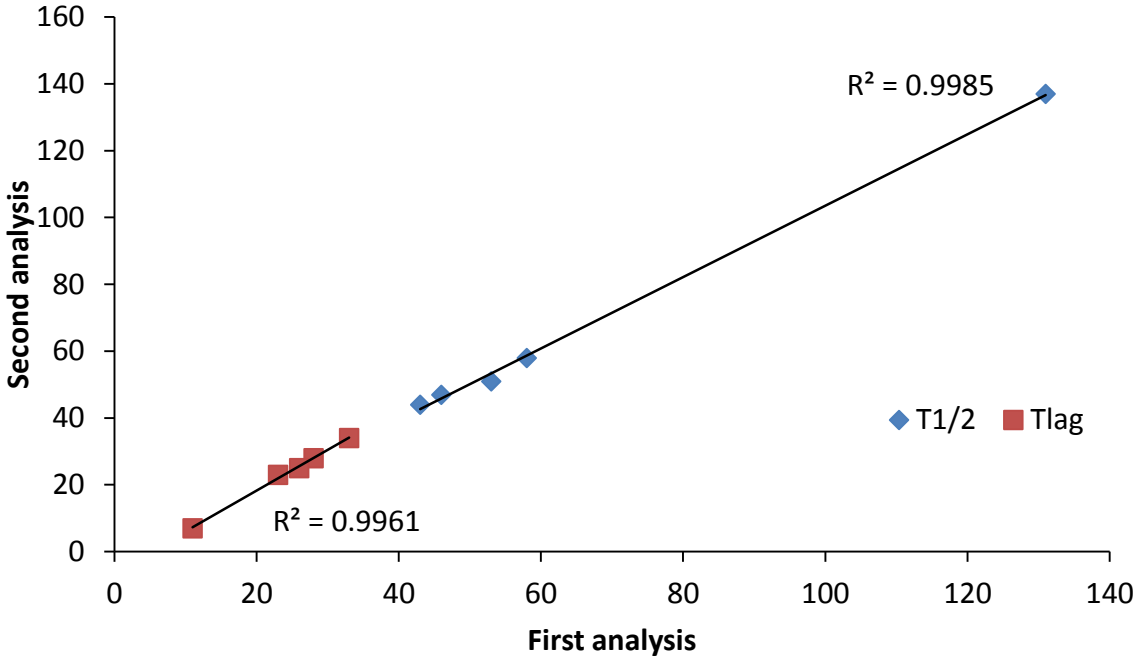

Figure A4. Correlations for $T_{½}$ and T_{lag} between first and second analysis

Conclusions

Results from this investigation show that breath samples stored and analysed up to 6 weeks post collection remain suitable for analysis and gastric emptying results remain highly reliable despite the concentration of CO_2 decreasing over time.