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Enhancing clinical nutrition, glycaemic, appetite and gastrointestinal responses of white rice

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Doctor of Philosophy

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List of abbreviations

ADA	American Diabetes Association
AgRP	Agouti-related peptide
Arc	Arcuate nucleus
AUC	Area under the curve
BMI	Body Mass Index
CNS	Central Nervous System
CONSORT	Consolidated standard of reporting clinical trails
CRF	Case Report Form
CVD	Cardiovascular disease
EEC	Enteroendocrine cells
EGI	Estimated glycaemic index
FAO	Food and Agriculture Organization of the United Nations
FDA	Food and Drug Administration
FOV	Field of view
FSE	Fast spin echo
GE	Gastric Emptying
GEVC	gastric excitatory vagal circuit
GEVMC	gastric excitatory vagal motor circuit
GG	Gellan Gum
GI	The Glycaemic index
GIP	Gastric inhibitory polypeptide or Glucose-dependent insulin polypeptide
GIT	Gastrointestinal tract
GIVC	gastric inhibitory vagal circuit
GIVMC	gastric inhibitory vagal motor circuit
GL	Glycaemic load
GLP-1	Glucagon-like peptide 1
GLUT2	Glucose transporter type 2
HAGG	High Acyl Gellan Gum

HASTE	Half-Fourier Single-shot Turbo spin-Echo
HbA1c	Glycated haemoglobin
HCl	Hydrochloric Acid
HI	The hydrolysis index
iAUC	incremental area under the curve
IDF	International Diabetes Federation
LAGG	Low Acyl Gellan Gum
LMIC	Low- and middle-income country
MMC	Migrating motor complex
MRI	Magnetic Resonance Imaging
NAc	Nucleus Accumbens
NAFLD	Non-alcoholic fatty liver disease
NPY	Neuropeptide Y
NSP	Non-starch polysaccharides
PFC	Prospective food consumption
PIS	Participant information sheet
POMC	Pro-opiomelanocortin
PP	pancreatic polypeptide
PYY	Peptide YY
ROI	regions of interest
RS	Resistant starch
SACN	Scientific Advisory Committee on Nutrition
SBWC	Small bowel water content
SD	Standard deviation
SEM	Standard error of the mean
SGF	Simulated gastric fluid
SGLT2	Sodium-glucose linked transporter 1
SI	Satiety index
SIF	Simulated intestinal fluid
SN	Substantia nigra

SPMIC	Sir Peter Mansfield Imaging Centre
SQ	Satiety quotient
SSF	Simulated salivary fluid
T1	Spin-lattice relaxation time
T1DM	Type 1 diabetes mellitus
T2	Transverse relaxation time
T2DM	Type 2 diabetes mellitus
TE	Echo time
TR	Repetition time
TTRTB	Time to return to baseline
UoN	University of Nottingham
VAS	Visual analogue scales
VTA	Ventral Tegmental Area
WHO	World Health Organization
WIGG	White Rice and Gellan Gum
α-MSH	α-melanocyte-stimulating hormone

Related publications and abstracts

Publications

- Syahrizal Muttakin, Serafim Bakalis, Peter J Fryer, Norah A Alshammari, Luca Marciani and Ourania Gouseti. Reducing starch digestibility of white rice by structuring with hydrocolloids. Food Research International 174, 113490, 2023.
  DOI: 10.1016/j.foodres.2023.113490
- Norah Alshammari, Moira Taylor, Rebecca Stevenson, Ourania Gouseti, Jaber Alyami, Syahrizal Muttakin, Serafim Bakalis, Alison Lovegrove, Guruprasad Aithal and Luca Marciani. Effect of intake of food hydrocolloids of bacterial origin on the glycemic response in humans: systematic review and narrative synthesis. Nutrients, 13, 2407, 2021.
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- Norah Alshammari, Katherine Riches, Syahrizal Muttakin, Ourania Gouseti, Serafim Bakalis, Alison Lovegrove, Guruprasad Aithal, Moira Taylor and Luca Marciani. The effects of adding gellan gum to white rice on the glycemic, gastrointestinal and appetitive responses: a randomised, controlled, crossover study. Current Developments in Nutrition. 6. 501-501, 2022.
  DOI: 10.1093/cdn/nzac077.004.
- Norah Alshammari, Syahrizal Muttakin, Liu Gingsu, Ourania Gouseti, Jaber Alyami, Alison Lovegrove, Guruprasad Aithal, Moira Taylor and Luca Marciani. (2021). The effect of adding gellan gum to white rice on the starch hydrolysis and glycemic index. Current Developments in Nutrition. 5, 571-571, 2021.
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Abstract

Background: Rice is staple food for over half the world's population, with approximately 480 million tonnes consumed annually. High consumption of white rice has been linked with high obesity rates and increased risk for type 2 diabetes. Controlling the properties of white rice products (e.g. reducing the glycaemic index (GI) and/or reducing appetite) with relatively simple interventions could contribute to producing foods with health-promoting profiles. One way to reduce the GI of white rice could be through processing. Addition of hydrocolloids has previously been shown to reduce the digestibility of foods. Hydrocolloids are polysaccharides used to increase viscosity and gelatinization and have many other applications in the food industry. Preliminary in-house data from *in vitro* studies suggested that the addition of 1% (weight over dry weight of rice) low acyl gellan gum (LAGG) during the cooking of white rice may reduce digestibility rates, though mechanisms are yet to be fully understood.

The overall aim of this project was to investigate for the first time the glycaemic and appetitive response to white rice meal cooked with LAGG in healthy adult participants.

Methods: Firstly, a static *in vitro* digestion study was undertaken to investigate the effects of adding LAGG to the rice cooking water on starch hydrolysis and GI. A dose response of 0%, 1%, 2% and 3 % LAGG (weight over dry weight of rice) was investigated using a static *in vitro* digestion model.

Secondly, an acute intervention, randomised, controlled, cross-over trial in 12 healthy participants was carried out. They attended two visits with one week washout having fasted overnight. They were asked to consume a dish of isoenergetic (232 kcal) jasmine white rice meal cooked with and without 3% (weight over dry weight of rice) LAGG and a glass of water. Postprandial blood glucose levels (using the finger prick method), appetite (using visual analogue scales) and gastric appearance and volumes (using magnetic resonance imaging, MRI) were monitored serially for two hours. Following that an *ad libitum* test meal was offered at lunch and food intake diaries were kept for the remainder of the day.

Lastly, a sustained intervention, randomised, controlled, cross-over trial in 8 healthy participants was carried out. The study consisted in a baseline visit similar to that described above measuring postprandial glucose and appetite responses and lasting for 3.5 hours. The participants were then asked to cook and consume at home the same rice test meal with or without LAGG once daily for 7 days and then returned for another study visit as described above. Food intake diaries and a pedometer tor record step count were provided to record a daily intake for the 7 days' home intervention. After 3 weeks' washout period they then returned to repeat the second arm of the study with the other allocated rice meal.

Key Results: The addition of LAGG affected rice starch hydrolysis in the static *in vitro* model of digestion. 3% LAGG significantly reduced the estimated GI value by 27%, from 94 for the control 0% LAGG, to 69 for the 3% LAGG samples ($P < 0.05$).

In the acute intervention study all 12 participants completed the protocol. The data showed that the incremental area under the curve (iAUC 2 hours) for blood glucose was significantly different between the meals ($P < 0.0001$). The composite appetite score AUC 2h was lower for the rice + LAGG meal than for the rice control but the difference was not significant. The MRI images showed that when rice was cooked with LAGG that were multiple rice boluses persisting throughout digestion time and that postprandial gastric volumes were lower compared to rice control though the difference from control was not significant.

In the sustained intervention study all 8 participants completed the protocol. The data confirmed an effect of LAGG in reducing blood glucose response compared to rice control at the baseline study (ANOVA $P = 0.0023$) and this was maintained after 1 week of sustained intervention (ANOVA $P = 0.0440$). After one week of eating white rice meal with LAGG the peak glucose value decreased by 1.2 mmol/L compared to the rice control meal ($P = 0.0219$). Differences in appetite were not statistically significant.

Conclusion

Modifying the cooking process of jasmine white rice with LAGG reduced the estimated *in vitro* GI of the rice in an *in vitro* model of digestion and reduced blood glucose responses in healthy humans both after an acute and a sustained intervention. The data are novel and add to knowledge in the field. The modification of the cooking process is safe, simple, and cheap and could potentially provide an intervention to help reduce the

post prandial glucose response to white rice, potentially impacting on the rising levels of obesity and type 2 diabetes seen in populations consuming white rice as a staple food.

1 Introduction

This chapter provides the background to the work undertaken. It describes the prevalence and consequences of obesity including the metabolic syndrome and type 2 diabetes mellitus (T2DM). The normal gastrointestinal and metabolic responses to meals including carbohydrate and regulation of appetite are considered. The concept of the glycaemic index (GI) is introduced. Rice and factors influencing the glycaemic response to its consumption, and the potential role in manipulating this response by changing the cooking process will then be considered.

1.1 Obesity definition, prevalence, risk factors and consequences, including T2DM.

The World Health Organization (WHO) defined obesity as an abnormal or excessive accumulation of body fat. The WHO classifies those living with obesity as having a body mass index (BMI) equal to or above 30 kg/m^2 and those living with overweight as having BMI equal to or above 25 kg/m^2 (1).

BMI is defined as the weight of an individual in kilograms divided by the square of their height in meters (kg/m^2). It can be used as a screening tool to classify people as living with underweight, a healthy weight, overweight, or with obesity. It is a practical tool for use in the general

population because it is easy to measure, and the categories are the same for both sexes. However, it has some limitations, and it should be regarded as a rough guide rather than a definitive measure of body fatness. Furthermore, it gives no indication of distribution of fat and use of the cutoffs in, for example, those with a high proportion of muscle are misleading unless it is used in conjunction with other measures, such as waist circumference (2).

Overweight and obesity prevalence has been increasing across the globe in both developed and developing countries (Figure 1.1). According to the WHO, in 2016, 39% of adults worldwide were living with overweight and 13% were living with obesity. Therefore, a total of 1.9 billion adults were living with overweight, and among these, 650 million were living with obesity. Moreover, the World Obesity Federation predicts that by 2030, one billion people worldwide will be living with obesity. The latest report from the World Obesity Atlas in 2022 shows that low- and middle-income countries (LMICs) have the highest number of individuals living with obesity. The numbers of people living with obesity have more than doubled across all LMICs and tripled in low-income countries compared with 2010 (3).

Overweight and obesity can lead to an increased risk of non-communicable disease (NCD) such as T2DM, hypertension, cardiovascular disease (CVD) and certain cancers, which all lead to an increased risk of premature mortality, morbidity and reduced quality of life (4).

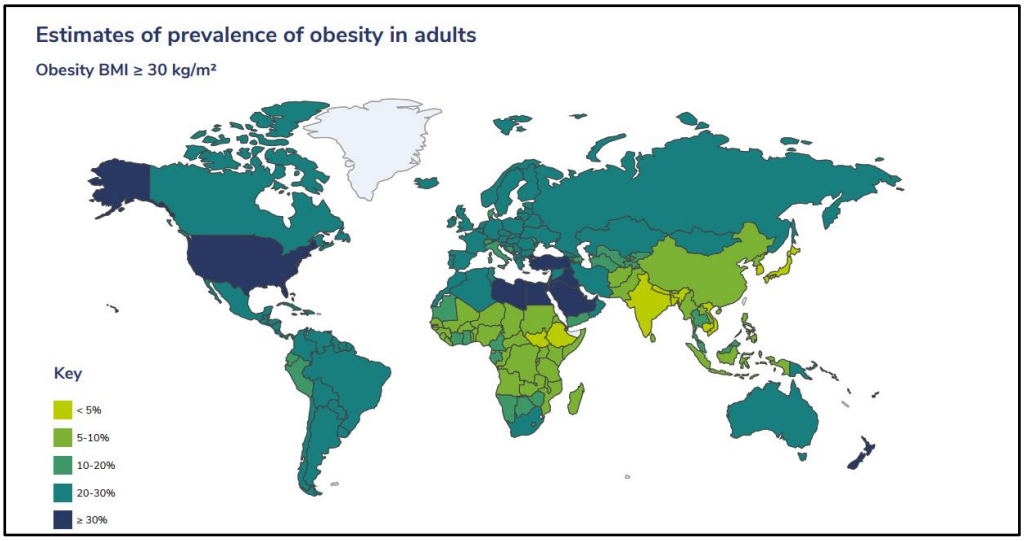


Figure 1.1 The estimated prevalence of obesity over the world (taken from https://gamapserver.who.int/gho/interactive_charts/ncd/risk_factors/) accessed March 2023).

Overweight and obesity result from an imbalance between energy consumption and energy expenditure in the body over a prolonged period. Genetic, environmental and other behavioural factors are commonly thought to increase the risk of the development of overweight, obesity and related negative health consequences such as T2DM. Important behavioural risk factors for obesity and related illnesses (such as T2DM), include low physical activity and the intake of energy dense, high- fat and/or high sucrose diets with high GI (5, 6).

In some individuals, a cluster of conditions are seen that are more common in those living with overweight and obesity. This is described

as the ‘Metabolic syndrome (MetS)’ and is known to increase the risk of cardiovascular atherosclerotic diseases (CVD), and T2DM. The main components are considered to be dyslipidaemia (elevated cholesterol and triglycerides and low high-density lipoproteins), high blood pressure and unregulated glucose level, obesity in the abdominal area and/or insulin resistance (7, 8).

1.2 Diabetes types, prevalence and causes

Diabetes mellitus is classified as type 1 (T1DM) and T2DM. T1DM is an autoimmune condition whereby atrophy of the acini in the pancreatic beta cells ~~that~~ leads to an insufficient insulin secretion response (9).

T2DM as defined by the American Diabetes Association (ADA), is a chronic condition that impairs the human body’s metabolism of glucose, leading (when untreated) to increased glucose levels in the blood, known as hyperglycaemia. Hyperglycaemia may result from insulin resistance and an inadequate insulin secretion from the beta cells of the pancreas. Dysfunction of the beta cells provides one of the main mechanisms involved in the development and progression of in T2DM (10, 11), the latter tending to be seen to a greater extent as the disease progresses (12). Those with glycated haemoglobin (HbA1c) ranges between 42 mmol/mol (6%) and 47 mmol/mol (6.4%) have an increased risk of developing T2DM (13).

Insulin resistance is a condition in which cells in the body, such as muscle, fat and hepatic cells, become less responsive to the hormone

insulin, which regulates the metabolism of glucose. When cells are resistant to insulin, one consequence is that they are less capable of absorbing glucose from the circulation, which may result in elevated blood glucose levels (14).

Increased age, obesity and low physical activity are all factors reported to promote insulin resistance and T2DM, particularly for people who are genetically susceptible. However the genetic abnormalities associated with the disorder are not fully understood (15, 16).

The International Diabetes Federation (IDF) reports that T2DM is the leading long-term metabolic disorder, accounting for at least 90% of all cases of diabetes mellitus worldwide. In 2018, about 387 million individuals were reported to have this condition and a predicted 179 million further individuals were undiagnosed. Around the world, about 2.1 million deaths are related to diabetes mellitus every year. Comorbidities are the reasons for death, including myocardial infarction, kidney failure, and stroke

T2DM increases the risk of acute complication such as diabetic ketoacidosis and diabetic coma, hypoglycaemia and hyperglycaemia. Chronic complications include macrovascular and microvascular disease. In addition to an increased risk of CVD and stroke, diabetic retinopathy (damage to the eye vessels), diabetic nephropathy leading to renal insufficiency and kidney failure, and microvascular damage to the foot are seen. (17). These factors lead to increased morbidity and premature mortality (16, 18).

An important approach to treatment and management is however lifestyle change intervention. This includes dietary changes, aiming to treat the underlying condition of insulin resistance, for example by weight loss promoted by decreased energy intake and increased physical activity, and to control blood glucose levels acutely, for example by prescribing a diet designed to minimise the glycaemic response. (19)

The Look AHEAD study conducted on 5000 patients was proposed to assess the impact of weight loss on health outcomes in individuals living with overweight and obesity and diagnosed with T2DM (20). After 1 year, an 8.6% weight loss was observed with the intensive lifestyle intervention, while the diabetes support and education group showed a weight loss of 0.7%. The higher weight loss group was associated with a significant reduction of HbA1c (20).

The IDF predicts that by 2035, more than 592 million individuals will have T2DM worldwide and that the majority of them (about 77%) will be living in LMICs. The aim of T2DM treatment is to maintain blood glucose levels within the normal range. Hypogylcaemic medication may be used including oral medication and injections such as insulin. There are a several antidiabetic agents and the principal classes are summarised in Table 1.1 (21, 22, 23).

Table 1.1 Principal classes of antidiabetic medication for type 2 diabetes mellitus and their key mechanisms. GIP, glucose-dependent insulinotropic peptide; (GIP). PPAR-γ, peroxisome proliferator-activated receptor-γ.

Categories	Example	Mechanism
Insulin sensitisers	Biguanides e.g. metformin	Reduce glucose production in liver, improve insulin sensitivity in peripheral tissues
	Thiazolidinediones e.g. PPAR-γ agonists	Reduce blood glucose levels, improve β-cells function, reduce insulin resistance
Insulin secretagogues	Sulphonylureas e.g. (1) tolbutamide, tolazamide, chlorpropamide, acetohexamide (2) glyburide, (3) glimepiride	Stimulate insulin release via a direct action on β-cells independently of glucose
	Short-acting type insulin secretagogues meglitinides, known as glinides	Control postprandial blood glucose level by increasing insulin secretion
Alpha-glucosidase inhibitors	Acarbose, miglitol, voglibose	Delay carbohydrates absorption in the small intestine. Thus, reduce postprandial blood glucose and insulin levels. Stimulate GLP-1 release from the gut
Incretin-based therapies	Subcutaneous injection of GLP-1 and GIP receptor agonists e.g. liraglutide, exenatide, dulaglutide, albiglutide, semaglutide	Stimulate β-Cells in the pancreas to release insulin. Inhibit glucagon secretion and hepatic glucose
Dipeptyl peptidase-4 inhibitors (DPP-4 inhibitors)	Oral incretin, mimic the actions of GLP-1. e.g. sitagliptin, vildagliptin, saxagliptin, alogliptin, linagliptin	Glucose-lowering agents that influence glucose-dependent insulin secretion, cause a delay in gastric emptying, increase levels of active GLP-1, decrease levels of postprandial glucagon, and reduce food intake
Sodium-glucose cotransporter 2 inhibitors	Phlorizin-based SGLT2 inhibitors e.g canagliflozin, dapagliflozin, empagliflozin, ertugliflozin	Decreased hyperglycaemia by improving urinary glucose excretion, block renal glucose reabsorption

1.3 Overview of the human gastrointestinal tract response to food

In order to understand how dietary approaches can be used in the treatment and management of T2DM it is important to understand the

normal anatomy and function of the gastrointestinal tract (GIT). This is shown in in Figure 1.2 and the digestive process are described briefly in this section.

1.3.1 Digestive system

The main function of the digestive system is the digestion and absorption of ingested food and beverages.

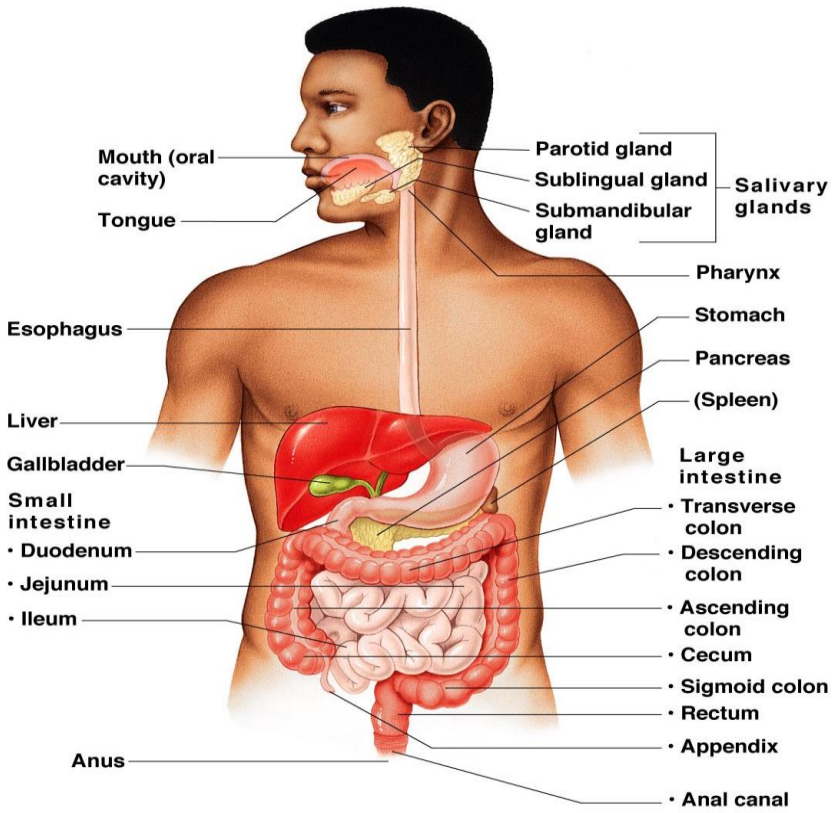


Figure 1.2 Anatomy of the human digestive system (taken from: https://anatomyandphysiologyi.com/digestive-system-overview/ accessed October 2019).

1.3.2 Food digestion

Food digestion and absorption comprise several phases: the cephalic / oral phase, the gastric phase and the intestinal phase. Throughout these phases, food undergoes five main processes in order to be utilised: ingestion, mechanical digestion, chemical digestion, absorption and excretion.

1.3.2.1 Cephalic and oral phase

Firstly, the sensory response to the sight, taste and smell of food promotes a physiological response which can include stomach acid secretion and gallbladder contraction. This is known as the cephalic phase (24).

When food enters the mouth, mastication breaks it into smaller food particles in preparation for swallowing, and at the same time, this process increases the surface area of the food. Saliva secreted from the salivary glands soften food to aid swallowing through the pharynx, and the salivary enzyme amylase begins hydrolysing carbohydrates in the mouth.

Hydrolysis is the splitting of one large molecule into two smaller units with the addition of water. Starch are hydrolysed into oligosaccharides: maltose, maltotriose and dextrins. Lipid digestion is also initiated in the oral phase by lingual lipase.

The bolus of food then moves from the mouth to the oesophagus propelled by the combined action of the tongue and the pharyngeal muscles. Peristalsis then promotes the movement of the bolus via the oesophagus to the stomach. Peristalsis is an involuntary movement of muscles in the form of contraction and relaxation waves through the GIT that helps to mix food with digestive secretions and pushes it through the GIT (25).

1.3.2.2 Gastric phase

The stomach is a J-shaped organ located in the upper left part of the abdomen. It comprises different anatomical regions. The proximal stomach is formed from the cardia, fundus and body. The distal stomach includes the antrum and the pylorus. In the stomach, food is mixed with gastric secretions (after which it is called chyme). The stomach contributes to the physical and chemical breakdown of the food. Signals from the stomach to the brain indicate gastric stretch/distention. detected by mechanoreceptor neurons in the walls (26).

The physical breakdown is carried out by contraction waves, which act to grind the food into particles. The chemical breakdown is achieved by the secretion of gastric hydrochloric acid (HCl) and the gastric enzymes pepsin and lipase. The stomach wall is protected from the acid by a gel-like mucous lining. The cardia contains mucous-secreting cells. The fundus in the stomach is lined with mucosa and presents thick folds called rugae. The fundus in the stomach serves as a food reservoir and

acts as a pressure pump. The proximal body and distal antrum constitute the peristaltic movement that function as a mixer.

The gastric glands in the stomach consist of mucus cells, chief cells and parietal cells and neuroendocrine cells. Mucus cells secrete mucus, whilst chief cells secrete pepsinogen and gastric lipase. Parietal cells secrete intrinsic factor and acid modulated by the gastrin hormone. Neuroendocrine cells also help the gastric acid production process (27).

The stomach does not secrete enzymes to digest carbohydrates but the activity of salivary secretions can continue in the stomach until a low pH level deactivates the salivary amylase. Gastric enzymes, such as pepsin, begin the chemical hydrolysis of protein, whilst gastric lipase continues the lipid breakdown (25). Chemically digested and mechanically reduced in size, the food particles then pass through the pyloric sphincter into the small intestine in the process of gastric emptying (GE).

1.3.2.3 Gastric motility

Gastric motility is defined as the movement of the stomach walls and is considered as an important contributor to the gastric digestion process. The gastric motility patterns of the proximal and distal parts of the stomach, following a meal, may vary. The contractions of the proximal part are tonic contractions, which are continuous with low frequency. By contrast, the contractions of the distal part of the stomach are stronger, phasic. The migrating motor complexes (MMCs) are cyclic motor contractions of the smooth muscle of the stomach, small intestine and

colon (28). The MMCs are increased during the fasting (interdigestive) state by the action of the hormone motilin. They have four distinct phases. Phase I is a period of quiescence. Phase II comprises patterns of low amplitude irregular contractions. Phase III provides the strongest bursts of contractions, travelling distally throughout the GIT. Phase IV is an intermediate period leading back to Phase I. The MMCs have an important role in cleaning indigestible remains in the GIT and preventing microorganism overgrowth in the small intestine. In addition, the phasic contractions are thought to be regulated by the interstitial cells of Cajal (ICC). The average frequency of phasic contraction the stomach is ~3 cycles/min as opposed to a frequency of 12 cycles/min in the duodenum.

Recent research has suggested that gastric motility is also regulated by the neural routes (28): the gastric inhibitory vagal motor circuit (GIVMC) and a gastric excitatory vagal motor circuit (GEVMC).

These are controlled by connected neurons, and jointly they establish the gastric inhibitory vagal circuit (GIVC) and gastric excitatory vagal circuit (GEVC). They are linked to anorexigenic and orexigenic neural pathways and controlled by different groups of neurons via vagal afferents. For example, when food is present in the GIT, hormones such as cholecystokinin and GLP-1, act to slow GE via the action of the GIVMC. In the inter-digestive state, ghrelin and motilin hormones increase GE via the GEVMC (29).

1.3.2.4 Gastric emptying and its regulation

GE is the movement of food from the stomach into the small intestine. The rate of GE of a meal is controlled by different variables and feedback mechanisms, including the meal composition, energy content and volume (30). The levels of macronutrients (carbohydrates, fat, and proteins) in the duodenum are sensed by receptors in the duodenal mucosa. The activation of these receptors is thought to initiate neural and hormonal responses to modify muscular tone and contraction frequency of the stomach and duodenum (31).

The physical characteristics of a meal, including its volume, can impact on GE rates (32). Kwiatek *et al.* reported that the GE rate was more rapid with a high meal volume and was slowed by a higher energy meal (33). Typically, the stomach empties at a rate of approximately 1-4 kcal/ min.

Another variable related to GE is the osmolality of the ingested meal or fluids. Osmolality refers to the concentration of active ions or molecules per litre solution of test meal or drink. The osmolality is modulated by various factors including the concentration of amino acids and carbohydrates. The osmolality of an isotonic drink or formula is 300 mOsm/L, which is similar to the osmolality of normal body fluids, approximately. The osmolality of beverages can modulate the gastric emptying, with higher-osmolality fluids slowing GE rates (34).

It has been reported that a liquid meal followed by a water drink empties approximately twice as fast in the first 35 minutes compared with a liquid meal with the same amount of water incorporated (35). It was shown in

a study using magnetic resonance imaging (MRI) that GE was delayed following a blending of a solid/liquid meal to a soup, which may promote increased satiety (36). Sieving is known as the splitting of solids and liquids in the stomach which can lead to quicker GE of fluids compared with solids. This gastric sieving can be avoided by blending the two fractions together to establish a homogeneous food (37).

1.3.2.5 Intestinal phase

When food reaches the small intestine, it is transported through three parts: the duodenum, jejunum and ileum. The small intestine is 6–7 metres in length. The digestive process continues aided by enzymes produced by the pancreas, and the absorption of nutrients and water mainly takes place in the small intestine (38).

Segmentation is a contraction of circular muscles in the small intestine that helps mix food with digestive secretions and aids in absorption.

The liver secretes bile salt, which is stored in the gallbladder and released into the duodenum which promotes emulsification of fat. The pancreas secretes enzymes to the duodenum through the pancreatic duct. Pancreatic α-amylase, a major carbohydrate digestive enzyme, continues starch hydrolysis. Oligosaccharides are hydrolysed by enzymes secreted from the cells lining the small intestine into short glucose monomers. Pancreatic lipase contributes to the digestion of fat, trypsin and protein (39).

The small intestine performs most of the carbohydrate digestion. The final process of carbohydrate digestion happens in the outer membranes of the intestinal cells. For example, each disaccharide is digested by specific enzymes and broken down into monosaccharides. Maltase enzyme converts maltose into two glucose molecules. Sucrose enzyme splits sucrose into glucose and fructose, and lastly lactase enzyme converts lactose into galactose and glucose. Monosaccharides (glucose, fructose and galactose) are the final products of digestion of both polysaccharides and disaccharides. Monosaccharides molecules can be absorbed by the capillaries of the intestinal villi. Monosaccharides are transported by the small intestinal epithelium via the action of various transporters such as the sodium glucose cotransporter 1 (SGLT1) and glucose and fructose transporters (GLUT2 and GLUT5) (40, 41). Thereafter, the monosaccharides reach the bloodstream and liver through the portal vein. After arriving at the liver, both galactose and fructose are then converted into glucose (40).

The large intestine is around 1.8 m in length and consists of four sections: the ascending colon, transverse colon, descending colon and sigmoid colon. After consuming a meal, all starch and glucose is fully digested within one to four hours, except for dietary fibre (described in more detail at Section 1.4), which remains in the GIT and is transferred to the large intestine, resulting in the formation of gas, water and short chain fatty acids (SCFA) (42). SCFA can provide health benefits, including anti-inflammatory and immunoregulatory activity.

The colon is mainly responsible for water reabsorption and fermentation of chyme. Excretion is the last step in the digestion process, in which undigested substances are stored in the form of stool and eliminated via the rectum and anus (38).

1.4 Carbohydrate classification

The pattern of digestion and absorption of carbohydrates depends on the characteristics of the carbohydrate consumed. Carbohydrates are composed of carbon, oxygen and hydrogen. Various methods of classifying and sub classifying carbohydrates have been suggested resulting in different, sometimes overlapping, groupings. The Scientific Advisory Committee on Nutrition (SACN) (43), suggested a classification of carbohydrates based on their chemical characteristics into: (1) sugar, (2) oligosaccharides, (3) polyols and (4) polysaccharides Table 1.2.

The SACN report also refers to ‘free sugars’ as all monosaccharides and disaccharides added to foods by the manufacturer, cook or consumer, plus sugars naturally present in honey, syrups and unsweetened fruit juices (43).

Polysaccharides, which are large molecules, contain chains of monosaccharides. Polysaccharides are sub-classified into starch and non-starch polysaccharide (NSP). Example of NSP are cellulose, hemicellulose, pectins and hydrocolloid gums (43). Example of starch are amylose and amylopectin. Starch can be found in almost all kinds of grains, such as rice, wheat, corn, millet, rye, barley and oats. Starch is

also found in different legumes. A single molecule of starch consists of more than 100 glucose molecules linked together as two types of polysaccharide: amylopectin, which has occasionally branched chains, and amylose, which has unbranched chain (44).

Starch that are not digested in the small intestine are known as resistant starch (RS). Starches in cereals may be not digested if they are not accessible to enzymes or if polymers amylose and amylopectin re-form ordered structures (45). Most carbohydrates (95%) are absorbed in the small intestine; these are known as glycaemic carbohydrates. Conversely, carbohydrates that are not hydrolysed by enzymes in the small intestine are called non-glycaemic carbohydrates (46).

The grouping ‘dietary fibres’, includes dietary components with a diverse range of physical characteristics, and in some cases they may provide an alternative form of energy to the human body following fermentation, predominately in the colon. The SACN report (43) recommends that dietary fibre should be defined as “all carbohydrates that are neither digested nor absorbed in the small intestine and have a degree of polymerisation of three or more monomeric units, plus lignin”. The definition thus incudes NSPs from plants and RS (43).

Table 1.2 Chemical classification of carbohydrates based on the SACN 2015 report (43).

Class	Subcategory	Consist of
Sugar	Monosaccharides	Glucose - fructose - galactose
	Disaccharides	Sucrose – lactose - maltose
Polyols		Sorbitol-mannitol
Oligosaccharides	Malto-oligosaccharides	Maltodextrins
	Non-digestible oligosaccharides	Raffinose, stachyose, fructo- oligosaccharides, verbascose
Polysaccharides	Starch	Amylose - amylopectin - modified starch
	Non-starch polysaccharides	Cellulose - hemicellulose - pectin - hydrocolloid gums

1.5 Carbohydrate metabolism and glycaemic response

Most of the cells in the body depend on glucose as their fuel source. Brain cells and the entire nervous system rely on glucose exclusively for energy. Hence, blood glucose homeostasis is crucial for the proper functioning of the body. To maintain this supply glucose must be available from either recently digested food or the liver (via glycogen breakdown or gluconeogenesis).

Glucose is stored as glycogen in the liver for many hours. Around 33% of the body's glycogen is stored in the liver and released when needed, and the remaining 67% is stored in the muscle cells. However, the latter

is utilised by the muscles when needed. A low amount of glycogen is normally found in the brain (47).

The glucose level in the blood is under endocrine control, with two of the key hormones being insulin and glucagon. The pancreas secretes insulin from beta cells. The primary role of insulin is to control the transport of glucose from the bloodstream into the body cells. A condensation reaction occurs in the cells of the liver to combine the glucose molecules and results in long branch chains of glycogen. Glucagon hormone enables the transfer of glucose from its storage to the blood. When glucose levels are low, the alpha cells in the pancreas release glucagon, which stimulates the release of glucose from the liver glycogen stores. These glycogen chains are broken down into individual molecules of glucose by hydrolysis reactions supplying glucose to the brain and other areas of the body during periods of fasting (48). In addition to insulin and glucagon, other hormones relevant to glucose metabolism are glucagon-like peptide-1 (GLP-1) and glucose-dependent insulin polypeptide (GIP). These hormones also have important roles in regulating appetite which will be further highlighted in the next section (49).

1.6 Appetite and food intake regulation

The appetite regulation system contributes to the process by which the body maintains a balance between energy obtained from food intake and energy expenditure by influencing eating behaviour and food intake. Appetite is defined as a general desire to eat and results from the

complex interplay of physiological, neural, hormonal, and psychological factors. Emotional state and stress can also influence appetite and subsequently eating behaviour (50).

1.6.1 The ‘satiety cascade’

The ‘satiety cascade’ presents a model of appetite and food intake regulation as shown in Figure 1.3. It refers to the series of physiological events that occur in response to food ingestion, leading to satiation, and subsequent food intake (51, 52). The term ‘satiation’ refers to the process during a meal leading to the termination of eating and thus controls meal size (within-meal or intra-meal inhibition). The term ‘satiety’ usually refers to the degree of satisfaction and/or fullness following food consumption. It leads to a decline in hunger and increase in fullness leading to inhibition of further eating after a meal is completed. This is also known as post-ingestive satiety or inter-meal satiety (53). Hunger is a subjective sensation or motivation to eat leading to meal initiation. It has been defined as the urge to eat arising from an energy deficit (54) and results from physical sensations such as a ‘rumbling stomach’. Hunger is defined by the Food and Agriculture Organisation (FAO) as “an uncomfortable or painful physical sensation caused by insufficient consumption of dietary energy” (55).

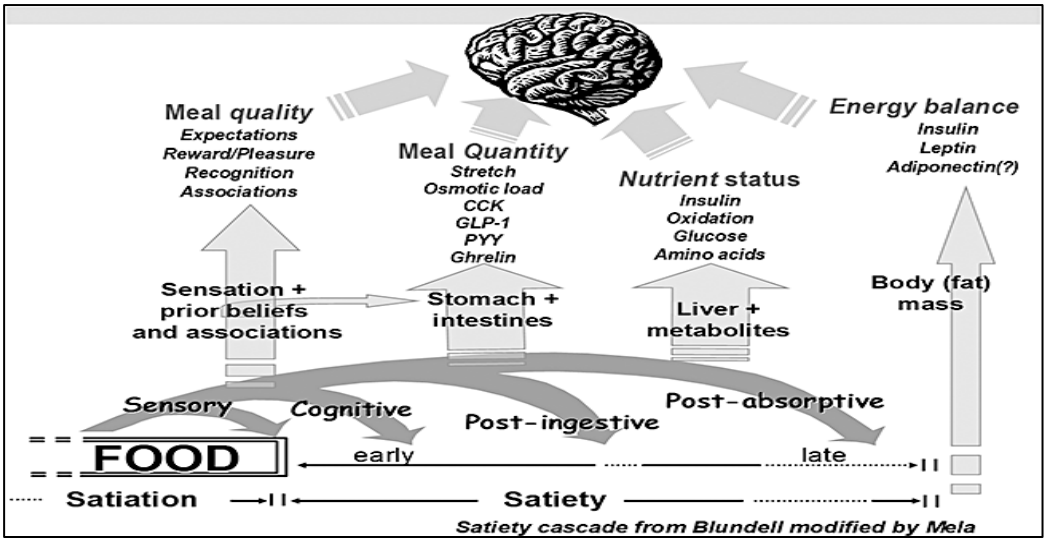


Figure 1.3 The Satiety cascade. This figure was taken Blundell J, De Graaf C, Hulshof T, Jebb S, Livingstone B, Lluch A, et al. Appetite control: methodological aspects of the evaluation of foods (53).

1.6.2 Homeostatic control /hedonic control of food intake and eating behaviour

Appetite and food intake regulation can be described in terms of the combined effect of two main systems: the homeostatic system and the hedonic system.

Homeostatic regulation is essential for basic metabolic processes and physiological needs as it controls the energy homeostasis via increasing the motivation to eat following a reduction in stored energy. When the energy stores are low in the body, hunger signals are triggered to encourage eating. Similarly, when energy stores are sufficient, satiety signals are activated to reduce the desire to eat. The homeostatic

pathway involves various neurotransmitters and hormones such as ghrelin, leptin, and insulin (56).

Hedonic eating, also known as reward-based pathway, refers to food intake for pleasure. It can overtake the homeostatic pathway via raising the desire to eat highly palatable food, which can lead to overeating. This in turn, can contribute to obesity development. Palatability is known as the pleasure or hedonic value related to food. It has been shown that highly palatable food can increase meal size intake in humans (57). The hedonic pathway affects eating behaviour and food choice. It involves neurotransmitters like dopamine, which play a role in reinforcing behaviours that lead to the consumption of foods (58). Both the homeostatic and hedonic pathways work together to regulate food intake, and interchange between these pathways can influence eating habits (57, 59).

1.6.3 Homeostatic regulation

In a response to the peripheral hormonal signals (for example arising from adipose tissue) and feedback from the GIT, the central nervous system (CNS) regulates appetite and eating behaviour. In the brain, the arcuate nucleus (Arc) of the hypothalamus is known to regulate appetite and food intake, and to maintain energy balance. The hypothalamus responds to various hormones and neurotransmitters to control hunger and satiety.

A group of specific neurons in the hypothalamus can release neuropeptides involved in the regulation of appetite. Orexigenic neuropeptides, such as Neuropeptide Y (NPY) and agouti-related peptide (AgRP), enhance appetite and increase food consumption. In contrast, another group of neurons can release anorexigenic neuropeptides. Pro-opiomelanocortin (POMC) neurons secrete α-melanocyte-stimulating hormone (α-MSH). This works on melanocortin receptors (mainly MC4R) to decrease the consumption of food (60, 61, 62).

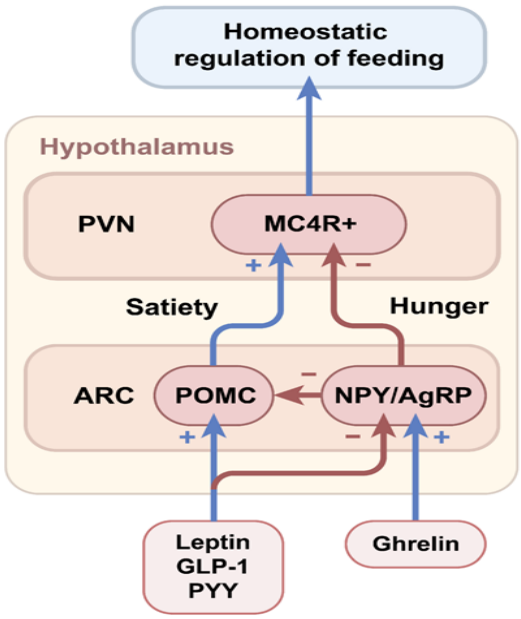


Figure 1.4. Schematic diagram of the homeostatic regulation of feeding in the hypothalamus. PVN: paraventricular nucleus of hypothalamus. ARC: the arc of the hypothalamus. MC4R: melanocortin-4 receptor. POMC: pro-opiomelanocortin NPY: Neuropeptide Y. AgRP: agouti-related peptide.GLP-1: glucagon-like peptide 1.PYY: Peptide YY. Taken from (63).

1.6.4 Brain reward /hedonic pathway

During the consumption of palatable foods dopamine, a neurotransmitter associated with pleasure and reward, is activated and released from the Ventral Tegmental Area (VTA) and substantia nigra (SN) located in the midbrain. This is responsible for generating pleasure sensation and motivation related to food consumption. These areas project into the nucleus accumbens (NAc) striatum, and orbitofrontal cortex (OFC). The NAc is part of the brain's basal ganglia and referred to as the brain's ‘pleasure centre’. When the NAc receives dopamine signals from the VTA, this will lead to the experience of pleasure creating positive feedback that encourages the consumption of the same foods again. Brain imaging studies support the concept that brain reward signalling can be involved in the development of obesity (64, 65).

1.6.5 Gut hormones

Hormones produced by the GIT and adipose tissue are essential in appetite regulation. The brain receives hormonal signals by circulation and via the vagal nerve (Figure 1.5). These hormones act as orexigenic hormones to stimulate appetite such as ghrelin and anorexigenic (anorectic) hormones to suppress appetite. The majority of satiation-inducing gut peptides also mediate their effects via vagal afferent nerves and these hormones have a key role in regulating GE and gut motility (66).

Enteroendocrine cells (EEC) are located throughout the GIT and pancreas. They have an important role in the gut–brain axis and produce more than 20 of the hormones that control different processes, including food intake, insulin secretion and gut motility. Examples of peptides released from intestinal enteroendocrine cells include Cholecystokinin (CCK) secreted from I-cells, GIP from K-cells, and GLP-1 and peptide YY (PYY) from L-cells (67, 68).

Some of the key hormones are briefly described below and summarised in Table 1.3.

Peripheral signals can be episodic and tonic. Episodic signals include orexigenic and anorexigenic peptides such as GLP-1, pancreatic polypeptide (PP), PYY, and CCK released from the GIT into the blood circulation and contribute to the short-term initiation and termination of the meal. Tonic signals, including insulin, leptin and adiponectin released from adipose tissue, show oscillations over longer term periods and reflect the amount of stored body fat (69).

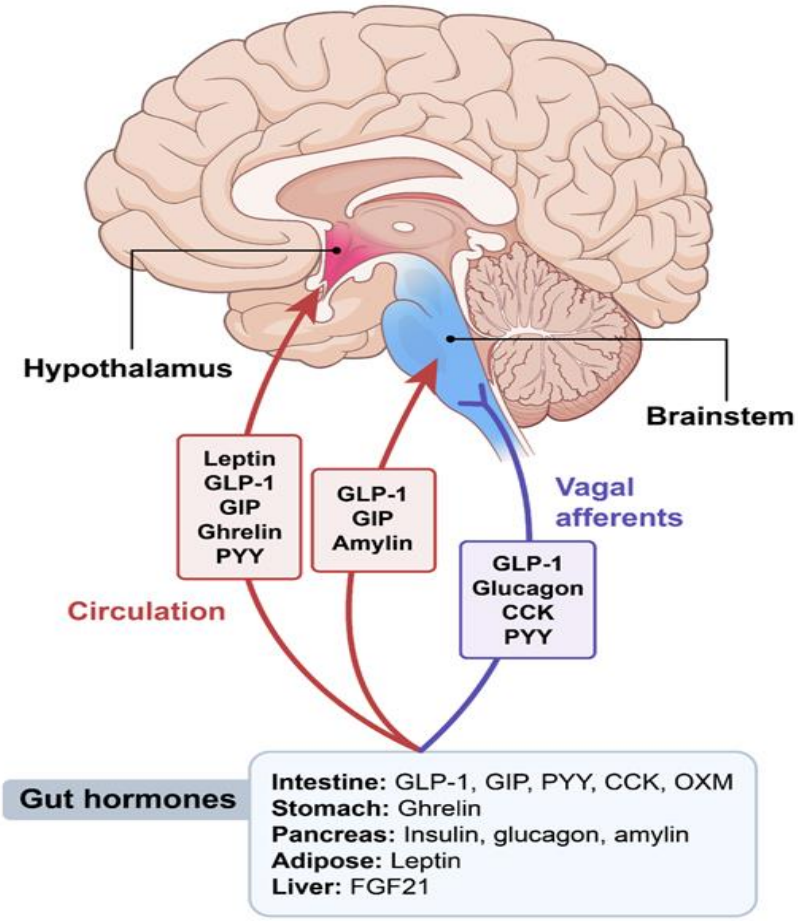


Figure 1.5. Diagram summarising some of the principal gut hormones and their action om the brain gut axis. Taken from (63).

1.6.5.1 Leptin

Leptin is a peripheral hormone produced by fat cells. It acts as a satiety hormone and reduces appetite when energy stores are sufficient. High levels of leptin inhibit NPY/AgRP neurons and activate POMC neurons leading to reduced appetite, while low leptin signalling will lead to increased appetite and food intake (70).

1.6.5.2 Insulin

Insulin is metabolic hormone secreted from β cells in the pancreas in response to blood glucose level and acts to control it. Insulin suppresses

the secretion of glucose in the liver. Plasma insulin is transported into the brain and leads to a reduction of food intake (71). Studies in humans suggest that Insulin is associated with appetite regulation, and the postprandial insulin level may act as satiety signal (72).

1.6.5.3 Amylin

Amylin is hormone secreted from the pancreas alongside insulin. It helps in the regulation of glucose levels and reduces meal intake by reducing appetite and slowing GE. It inhibits the secretion of pancreatic glucagon (71).

1.6.5.4 Glucagon

Glucagon is secreted from α cells in the pancreas. It helps to increase the secretion of glucose from the liver. It can signal to the brain via the vagal nerve and reduce meal intake (71).

1.6.5.5 Ghrelin

Ghrelin is a 28 amino acid peptide hormone released in the stomach fundus. Ghrelin is an orexigenic gut hormone and is often referred to as the ‘hunger hormone’. Ghrelin acts in the brain to control food consumption. The circulating ghrelin level is high during fasting and decreases after food intake (73, 74). It also acts in the stimulation of gut motility, GE and the secretion of gastric acid (75).

1.6.5.6 Glucagon-like peptide-1

GLP-1 is a 30 amino acid peptide, secreted from enteroendocrine L cells in the small intestinal in response to nutrients in a meal, such as

carbohydrates and fat (76). It contributes to satiety feelings and decreases food intake. GLP-1 slows the rate of GE and stimulates the secretion of insulin in the pancreas allowing the control of blood glucose levels (49).

1.6.5.7 Gastric inhibitory polypeptide

GIP, also called glucose-dependent insulinotropic polypeptide, is a 42–amino acid peptide secreted by K cells in the duodenum and jejunum. It acts to stimulate insulin secretion in the pancreas and increases satiety (77).

1.6.5.8 Pancreatic polypeptide

PP is a 36-amino acid peptide, secreted by F cells or PP cells in the pancreas. PP levels in the blood increase after a meal and act to increase satiety and reduce appetite. PP has an effect on gastrointestinal motility. It slows GE and the motility of the intestine, contributing to feelings of fullness after consumption of a meal (78).

1.6.5.9 Cholecystokinin

CCK is a peptide hormone released by I- cells in the duodenum and jejunum of the small intestine in response to food consumption. CCK was the first gut hormone reported to affect appetite (79). it contributes to increases in fullness and reduced appetite. CCK signals to the brain and slows GE (80). It stimulates pancreatic enzymes and acts on the gallbladder to release stored bile into the duodenum facilitating the digestion and absorption of fats (81).

Table 1.3 Summary of key hormones involved in the regulation of appetite, gut motility and glucose metabolism. Adapted from (82) and modified.

Hormone	Site of secretion	Major actions
Leptin	Adipose tissue	Anorectic Reduces hunger
Insulin	Pancreatic β cells	Decreases blood glucose
Amylin	Pancreatic β cells	Anorectic Inhibits gastric secretion Slows gastric emptying Reduces blood glucose
Glucagon	Pancreatic α cells	Anorectic Gluconeogenesis Glycogenolysis
Ghrelin	Gastric fundal A cells	Orexigenic Increases gastric motility Growth hormone release
Oxyntomodulin (OXM)	Gastrointestinal L cells	Anorectic Glucose dependent insulin release Slows gastric emptying Vagal and CNS effects
Glucagon-like peptide-1 (GLP-1)	Gastrointestinal L cells	Anorectic Glucose dependant insulin release Slows gastric emptying Vagal and CNS effects
Glucose-dependent insulinotropic polypeptide (GIP)	Gastrointestinal enteroendocrine K-cells	Anorectic Stimulates insulin secretion Delays GE
Pancreatic polypeptide (PP)	Pancreatic PP cells	Anorectic Delays GE
Cholecystokinin (CCK)	Intestinal I cells	Anorectic Gall bladder contraction Slows gastric emptying Pancreatic enzyme secretion
Peptide YY (PYY)	Gastrointestinal L cells	Anorectic Slows gastric emptying Vagal and CNS effects

1.6.5.10 Peptide YY

PYY is a 36-amino acid peptide, released from the L cells in the GIT in response to the consumption of food. It is considered a satiety peptide as it acts on the brain to reduce appetite and food intake. Levels of circulating PYY are low during fasting and increase in response to food consumption (83).

1.6.6 Role of macronutrient composition on appetite

The dietary macronutrient composition of a meal can influence satiety and the release of gut hormones. The mechanisms by which different macronutrient effect satiety and energy intake are complex and differ amongst different macronutrients. Under isoenergetic meal conditions, the macronutrients show a hierarchy for impact on satiety. Protein shows a stronger effect on satiety followed by carbohydrates and then fat (84).

Foster-Schubert *et al* measured ghrelin levels for three different isocaloric drinks of 500 mL given during three visits. One drink contained 80% of its energy as carbohydrate, one drink contained 80 % of its energy as protein and the final drink contained 80% of its energy as fat. In the 16 participants, ghrelin levels dropped immediately after the ingestion of the 3 drinks. The total ghrelin level decreased more after the predominantly carbohydrate- and protein-containing drinks than after the predominately lipid-containing drink. The ghrelin level was suppressed by 30% in the predominately carbohydrate drink compared with 21% in the predominately fat-containing drink ($P = 0.011$), while ghrelin level for

the predominately protein-containing drink was suppressed by 28% compared to 21% in the fat drink, *P* = 0.017 (85). Given that there was no significant difference in suppression of ghrelin between protein and carbohydrate, this suggests that factors in addition to ghrelin may explain the hierarchy, as noted by Poppit *et al* with respect to satiety (84).

It is reported in several studies that the macronutrient composition of meals can mediate the secretion of post-prandial hormones. For example, a low carbohydrate/high fat meal resulted in a 55 % greater level of postprandial PYY serum level compared with a low fat/ high carbohydrate meal (*P* = 0.005) (86).

Van der Klaauw *et al.* conducted a three-way crossover study on eight healthy participants to evaluate the effect of three different breakfast meals high in carbohydrates, fat, and protein on postprandial levels of GLP1 and PPY. The results showed that after ingestion of the protein breakfast, the PYY level was the highest compared with the high fat breakfast (*P* = 0.011) and the high carbohydrate breakfast (*P* = 0.012). Also, GLP-1 levels were shown to be higher following the consumption of the high protein breakfast compared with the other breakfast meals (87).

1.6.7 Methods for measuring appetite response.

Visual analogue scale (VAS) ratings are a standard tool for assessing subjective attributes of appetite and are often used to quantify hunger, satiety, desire to eat and prospective food consumption (PFC) (88).

Based on the analysis of 23 randomised controlled trails, a change in appetite VAS rating of ≥15-25 mm on a 100 mm scale is considered to be the minimum effect corresponding to a significant difference in the subsequent energy intake (89).

Satiating efficiency (90), satiety index (SI) (91) and satiety quotient (SQ) (92) have also been proposed. The SQ is used to describe the level of satiety following consumption of a meal (93). It has been reported that that VAS ratings are good predicators of free living energy intake and that SQ can predict fullness (94). VAS and SQ are described in more detail in the methods chapter (Section 2.3.2).

1.7 Glycaemic index and glycaemic load

A useful tool for predicting the body's glycaemic response to a meal is the 'GI' of the constituent foods. The principle of GI was first introduced by Jenkins et al in 1981 (95). It reflects the postprandial rise in blood glucose following consumption of a sample of food with a known amount of carbohydrate, relative to a standard containing the same amount of carbohydrate.

The GI thus acts as a ranking system that measures blood glucose level change with a specific food compared with white bread or glucose which are given a value of 100 (96). It is established by feeding 10 or more healthy participants 50 grams of available carbohydrate and by measuring blood glucose, usually within a two-hours interval (97).

Foods have been classified based on this concept as being ‘low GI’ if they have a GI of 55 or below, ‘medium GI’ if between 56 and 69 or ‘high GI’ if they have a GI of 70 or above. The use of GI can be a useful tool for patients diagnosed with T2DM. There have been several studies that examined the association between the GI of foods and postprandial glycaemic response. Consumption of low GI foods has been shown to result in a lower glycaemic response (98, 99, 100). Foods with low GI are thought to increase blood glucose gradually (101). It has also been suggested that a low GI diet can reduce HbA1c and indeed fasting glucose (102). In contrast, higher GI foods are associated with a higher insulin response as high-GI foods are absorbed quickly, and this in turn can cause a rapid increase in blood glucose levels (101) and increase the risk of T2DM (103). A randomised crossover study showed that high GI foods given over a period of week significantly increased liver fat compared with when the same participants were given a low GI food. This suggests that low GI foods could be a possible intervention to prevent or manage the prognosis of the disease (104).

However, a food with a low GI can still have a significant impact on blood glucose levels if it contains a high amount of carbohydrate. Therefore, the concept of glycaemic load (GL) was introduced to take into consideration both the GI and the amount of carbohydrate in a meal. GL can be calculated using the following equation [1.1]

Glycaemic load (GL) = GI% × grams of carbohydrate per serving [1.1]

1.8 Rice

Rice is an important factor in food security for many countries. It is a staple food for around half of the world's population, and its consumption is estimated to be 480 million tonnes per year (105). As around 870 million people globally are expected to suffer from chronic malnutrition, increasing rice production could help fill this gap. According to the FAO, rice is widely grown in Asia, the Pacific region, parts of Latin America and the Caribbean, and, increasingly, in Africa. Rice plays an important role in the nutrition of these countries. Additionally, rice is a source of income for more than 200 million families in developing countries (106). The production and consumption of rice is highest in Asian countries, as rice supply forms up to 50% of their total dietary requirements.

1.8.1 Rice production

Whilst the population of low-income countries increased by 90% between 1966 and 2000, paddy rice production increased by 130% in the same period (107). Moreover, the International Rice Research Institute (IRRI) reported that milled rice production increased from 150 million tonnes in 1960 to 450 million tonnes in 2012. Figure 1.6 shows the FAO rice Market Monitor. Rice is grown in over 100 countries that produce more than 715 million tonnes of paddy rice annually. Antarctica is the only continent in which rice is not grown. Fifteen countries account for 90% of the world's rice harvests. China and India alone account for 50% between them, and most of the rest (40%) is produced by Indonesia, Bangladesh, Vietnam,

Myanmar, Thailand, the Philippines, Japan, Pakistan, Cambodia, the Republic of Korea, Nepal and Sri Lanka. Other major non-Asian rice producing countries account for 5% of the rice produced globally, including the United States, Brazil, Egypt, Madagascar and Nigeria (106).

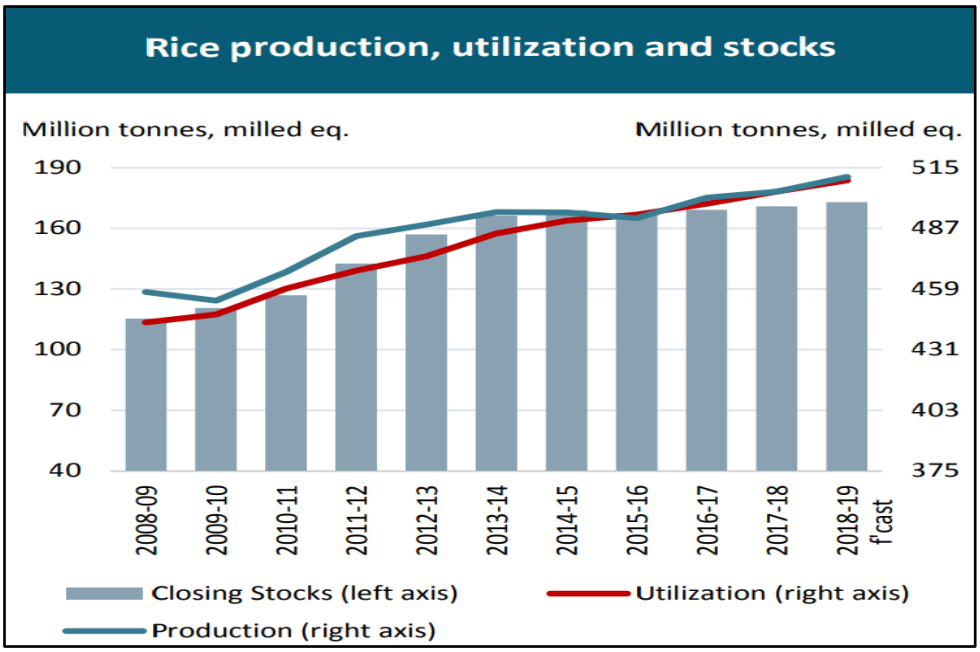


Figure 1.6 The Food and Agriculture Organisation of the United Nations Rice Market Monitor, Volume XXI Issue No. 1, April 2018 https://www.fao.org/3/I9243EN/i9243en.pdf.

1.8.2 Rice types and cultivation

Rice is from the genus *Oryza,* which includes 21 species. Only two species are cultivated and consumed by humans: *Oryza sativa* and *Oryza glaberrima. Oryza sativa* originated 8,000–15,000 years ago in Southeast Asian countries, such as Myanmar, Thailand, North Vietnam and China. *Oryza glaberrima* is believed to be cultivated from its wild

precursor *Oryza Barthii,* developed in West Africa by the people of tge River Niger around 3,000 years ago.

O. sativa is more widely grown in Asia, North and South America, Europe, the Middle East, and Africa, while *O. glaberrima* growth is limited to Africa; however, it is rapidly being replaced by *O. sativa.* Most rice types were developed from *Oryza sativa. Indica, japonica and javanica* are the three sub-species of *Oryza sativa,* with *indica and japonica* being the most common.

Basmati and Jasmine rice are well known types of rice both belonging to *indica* variety*. Indica* has long grains and grows mainly in tropical and subtropical regions in Asia, as it is drought tolerant but not tolerant to cold weather. The grain is medium-long, narrow and flat. *Japonica* is known for its short, wide and sticky grains. It is tolerant to cold temperatures, but not to drought, insects or diseases. *Javanica* has a tall plant and a medium grain size. Figures 1.7 and 1.8 show the rice types and species (106).

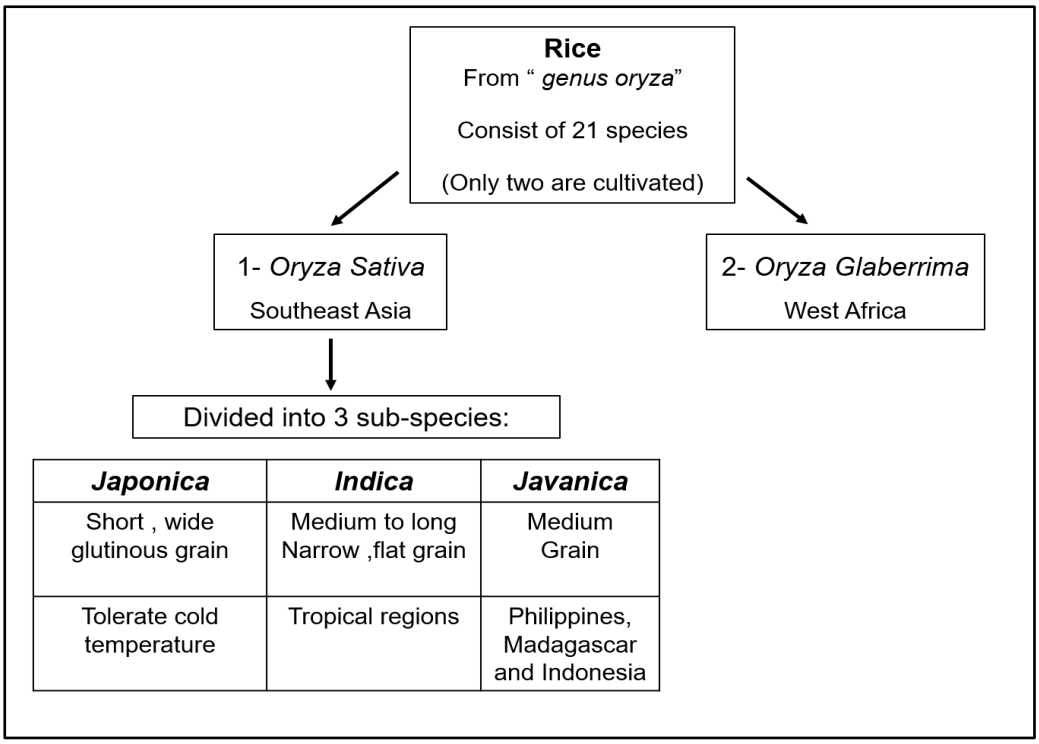


Figure 1.7 Rice from *genus oryza* types and species.

Figure 1.8 The appearance of non -milled *Oryza sativa* sub-species *Japonica, Indica and Javanica.* (Taken from http://www.knowledgebank.irri.org/ericeproduction/grains_3_races.jpg accessed October 2022).

1.8.3 Methods used to process rice.

Paddy rice, or rough rice, is the final product of the harvesting and threshing (separation) of rice grains. Paddy rice consists of 1) an outer husk layer, also called hulls; 2) germinated bran, which contains fibres and minerals; 3) the endosperm, which contains starch (white rice); and 4) the germ, which is the embryo containing oil and vitamins (Figure 1.9). Most rice varieties are composed of approximately 20% rice husk, 11% bran and germ layers, and 69% starchy endosperm, which is referred to as ‘milled’ or ‘white’ rice.

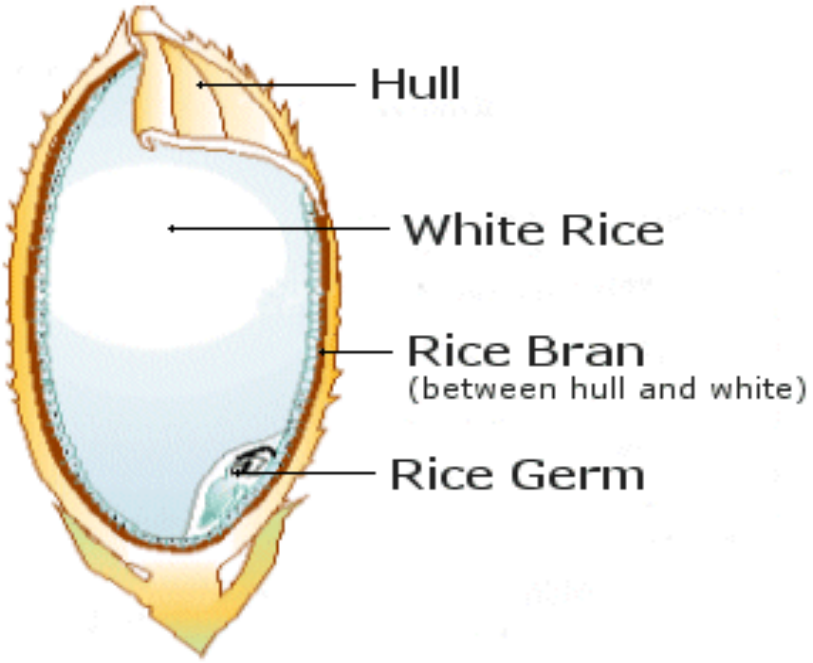


Figure 1.9 The Structure of the rice grain kernel. (taken from: http://agritech.tnau.ac.in/postharvest/thump/rice_structure.gif /accessed October 2022).

Milling is an important process whose main goal is to produce edible rice. There are several types of milling. The removal of the surface husk layer only produces brown rice, and the removal of both the husk and bran layers results in white rice (108).

1.8.4 White rice and health

White rice consumption has been linked to an increased risk of NCD including obesity, T2DM and the metabolic syndrome (109). High consumption of white rice has been linked with high obesity rates and an increased risk of T2DM (110). A cross-sectional study conducted on 415 female adolescents reported a significant association between the intake of rice and obesity (111).

Consumption of high energy refined carbohydrates such as white rice may have contributed to the rapid increase in T2DM rates (112, 113). A number of studies have reported that an increased intake of white rice is related to increased T2DM in various countries of the world. A study by the Japanese Public Health Centre showed that women had a 65% higher diabetes risk when consuming > 437 grams of cooked white rice per day compared to < 287 grams of the same rice (114). Villegas *et al.* (115) reported a 78% increased risk of T2DM when the intake of white rice exceeded 300 grams per day compared to only 200 grams of the same rice.

A meta-analysis conducted on seven prospective cohort studies in Asian and Western populations, with follow-up visits between 4 years and 22

years, showed that one additional serving of white rice per day could increase the risk of T2DM by 11% (116).The same study also found that groups of people with a high consumption of rice had a 27% higher diabetes risk than the low consumption group (116). This effect was observed (55% increase) in Asian individuals compared to Western populations, as the former consume large amounts of rice. Another meta-analysis study reported a 23% increased risk of diabetes with each additional serving of white rice per day (117).

1.8.5 Factors affecting the GI of rice

Different rice types have different GI values. Jasmine rice, for example, has a high GI between 96 and 116 while Basmati rice is considered to have a lower GI (118).

The GI of rice is influenced by several factors. A systematic review (119) examined the influence of the rice processing method on insulin responses and postprandial glycaemia and found that the main factors are the starch type (amylose and amylopectin), processing (particularly parboiling and consumer processing), degree of starch gelatinisation, storage and reheating (119).

1.8.5.1 Starch type (amylose and amylopectin)

Starch from plant foods contains amylose and amylopectin polysaccharides, and the ratio of amylose to amylopectin can influence

the degree of starch digestion. Amylose contains α-(1,4) bonds between glucose monomers making a linear polymer, whilst amylopectin contains α-(1,4) bonds and α-(1-6) bonds resulting in a highly branched structure. The structures of amylose and amylopectin are shown in Figure 1.10

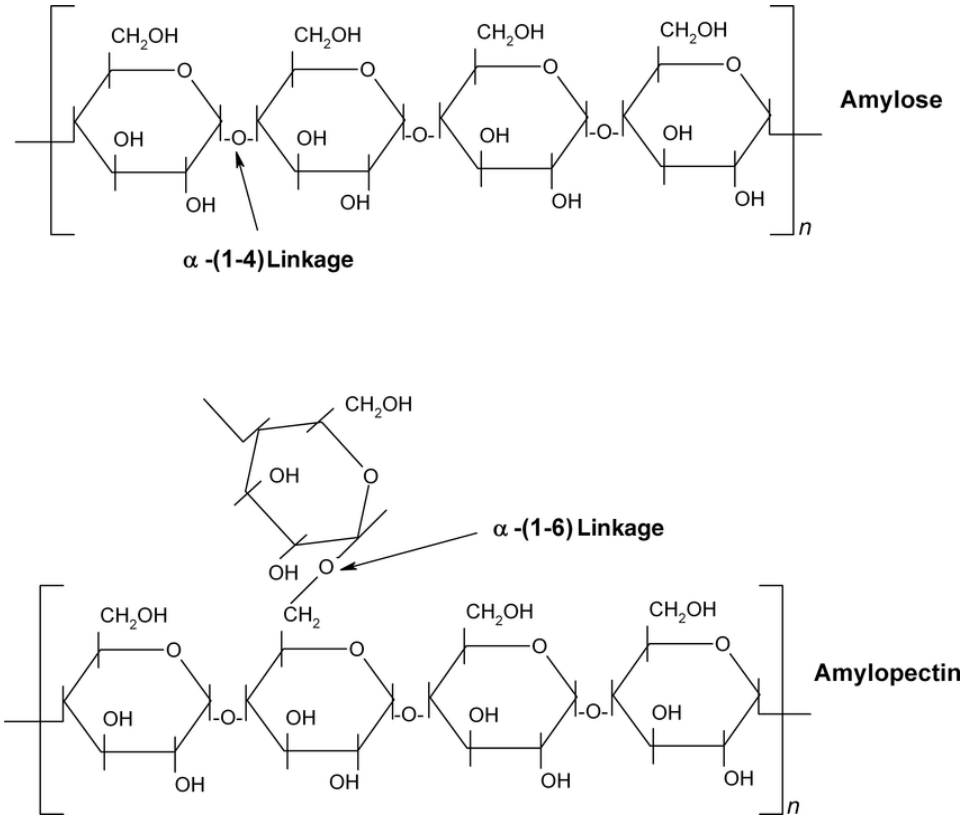


Figure 1.10 Chemical structures of amylose and amylopectin. Taken from (120).

The amylose content influences the GI: the higher the amylose content in rice, the lower the GI. Amylose is digested at a slower rate compared with amylopectin because the compact linear structure of amylose is less accessible to digestive enzyme α-amylase (121). Therefore, starch with high amylose content needs slightly prolonged digestion time. Behall *et*

al. conducted a study on 25 subjects and tested a bread with 70% versus a bread with 30% amylose. They reported that the postprandial 2-hour glucose area under the curve (AUC) was significantly lowered following the consumption of bread containing 70% amylose compared to 30% of the starch being amylose (122).

Miller *et al.* tested low and high amylose rice on healthy volunteers and reported lower glucose and insulin levels in the high amylose group than in the low amylose group (123, 124).

The consumption of a starch food high in amylose could confer a health advantage to people with an impaired glucose response such as those with pre-diabetes and those with diabetes. Nonetheless, amylose alone is not considered a good predictor of the rate of starch digestion or glycaemic response (125). A randomised study reported a lower blood glucose concentrations in individuals who consume high amylose rice compared with standard rice (126). An *in vitro* study of rice noddle demonstrated a lower GI with the high amylose rice (127).

1.8.5.2 Cooking

Different varieties of rice with the same amount of amylose can vary in gelatinisation specifications. Starch gelatinisation is the process whereby starch and water are exposed to heat, resulting in the starch granules expanding and swelling. This can impact the digestibility of the starch and the glucose response in the blood. The gelatinisation of rice is affected by the level of cooking and the amount of water used. This

directly influences digestibility and the glycaemic response. Boiling rice for only 5 minutes leaves the grains more intact, which results in a low GI, while boiling rice for 15 minutes splits the grains and makes them swell, increasing the GI of the rice (128). The cooking strategy, duration of cooking and cooking fluid volume all affect the GI of the rice.

1.8.5.3 Soaking

Soaking before cooking is one rice preparation technique. Basmati rice is the most common pre-soaked type; pre-soaking expands the grains and produces a more desirable product. Soaking was found to change GI in rice.

1.8.5.4 Processing

There are several rice processing methods, such as puffing, extrusion and explosion. Extrusion is the application of high pressure and temperature to gelatinise starch, sometimes followed by drying. Extrusion has been shown to reduce both *in vitro* starch digestion and blood glucose responses in healthy volunteers and people with diabetes (129). Another important process is parboiling, which occurs when the starch of the rice grains is gelatinised and retrograded as a result of a hydrothermal treatment. Parboiling lowered the GI and increased the RS content of rice (130).

Ranawana *et al.* reported that easy-cook basmati rice (instant rice), usually pre-gelatinised, has a higher GI than original basmati rice (118). This might be related to the extended duration of cooking, resulting in a higher degree of starch gelatinisation.

1.8.5.5 Cooling

Cooling cooked rice reduces GI through retrogradation. Starch retrogradation is a reaction that occurs when the amylose and amylopectin chains are cooked and crystallised. The highest crystallisation rates of rice have been observed at around 4°C (131). *In vitro* studies have found that keeping cooked rice at a temperature of 4°C (refrigerator temperature) for 24 hours leads to a reduction in the digestibility and GI level for both brown rice and milled rice (132). Cooling appears to be a simple, effective way to lower the GI of rice.

1.8.5.6 Fibre

A study reported that the rice bran fraction in brown rice lowered the glycaemic response in study participants with diabetes (133). However, it is remains unclear whether fibre has a direct influence on the GI of rice. Brown rice may have a lower GI than white rice due to other related reasons, such as lower digestibility or higher RS levels and bran content.

1.8.5.7 Particle size

The particle size of food has been shown to affect the digestibility of starch in both *in vivo* and *in vitro* studies. Cooked ground rice leads to significantly higher glycaemic responses than cooked whole rice grains in both normal volunteers and those with diabetes (134). Ranawana *et al*. investigated individual variations in mastication degree and GI, and people who chewed rice more had a higher glycaemic response than those who chewed less (135, 136). The GI is influenced by the particle size of the rice consumed. According to the study, digestion rate in the small intestine is determined by the extent of rice breakdown during chewing.

1.8.6 Use of hydrocolloids to modify GI of rice

In the literature a different method of modifying the GI of rice through processing was proposed. This involved cooking the rice with the hydrocolloid Xanthan, which resulted in a reduction of the GI and, in turn, of the blood glucose responses in human participants (137). This is an interesting approach which is worth exploiting in more detail, starting with an exploration of potential hydrocolloids.

1.9 Food hydrocolloids

The term ‘hydrocolloid’ is obtained from the Greek *hydro* (water) and *kolla* (glue). Hydrocolloids are polysaccharide gums used in the food industry to improve the functional properties of food, such as thickening

and changing viscosity, foaming, emulsification and stabilisation. They have various uses in this sector. Food hydrocolloids are well known for their ability to hold water, which is important for mixing different food substances. They are used in several food products, such as ice cream and infant food (138). Examples include inulin, guar gum, and gum Arabic (Figure 1.11).

Beside their use in the food industry, hydrocolloids have uses in medical nutrition too. For example they are used for patients requiring texture modification due to dysphagia to ensure a safe swallow. Diets for those with dysphagia may require increased viscosity to avoid the flow of food into the respiratory tract instead of the oesophagus. Hydrocolloids can also be added to infant formula. Infants who have regurgitation problems may be fed formulas thickened with hydrocolloids (139).

Food hydrocolloids can be categorised into two main groups: as thickening agents to increase viscosity - such as xanthan gum, locust bean gum, carboxymethyl cellulose and guar gum, and as gel-forming agents - such as agar-agar, carrageenan, gelatin, pectin, gellan gum (GG), and konjacglucomannan. Food hydrocolloids can also be classified based on their natural source as shown in Figure 1.11 (138).

1.9.1 Food hydrocolloids and health

Adding hydrocolloid gums can have potential health benefits; for example their addition can lower food digestibility by 10% compared with foods without polysaccharides (140) in turn affecting their GI. Ikegami *et al.*

also reported that hydrocolloids altered the pattern of digestion of food; for example, in patients with diabetes, guar gum reduced the postprandial blood glucose response, which led to a decrease in the concentration of urine glucose (141, 142).

Gelatin, which is another hydrocolloid type, resulted in a decreased postprandial blood glucose levels by flattening the absorption pattern for digested starch (143). The addition of carboxymethyl cellulose was found to decrease corn starch hydrolysis of the food matrix (144).

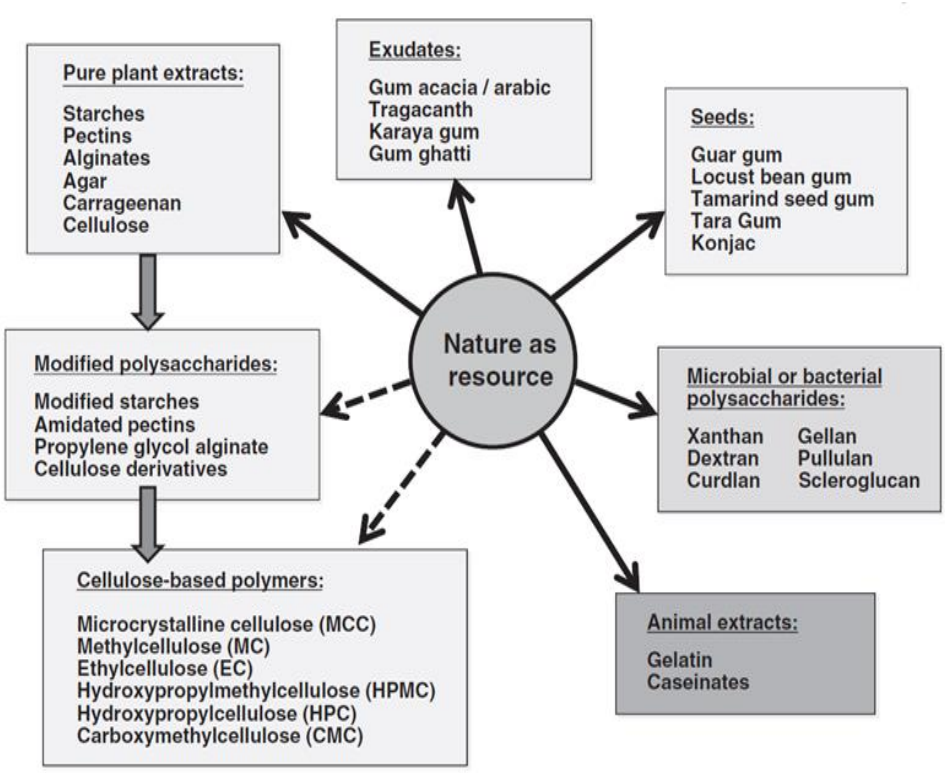


Figure 1.11 Classification of different types of hydrocolloids gums currently used by the food industry. The classification is based on the original natural sources of the different hydrocolloids. Figure taken from (138).

A possible explanation for the role of hydrocolloids in the inhibition of starch digestion may be due to the formation of a physical barrier that limits digestive enzyme access (145, 146).

Schneeman *et al.* attributed the inhibitory effect of hydrocolloids on protein hydrolysis to the direct binding of hydrocolloids themselves to the enzyme, thus slowing the interaction between the enzyme and the substrate (147).

Previous work (148) evaluated *in vitro* digestion of rice preparations containing a range of hydrocolloid gums up to a concentration of 1% weight/dry weight of rice: low acyl gellan gum (LAGG), high acyl gellan gum (HAGG), gum arabic and pectin. This work suggested that LAGG slowed down starch hydrolysis, whilst starch hydrolysis in the presence of the other gums was comparable to the controls. Considering these results, it was decided to focus on LAGG for the work in this thesis. A more detailed description of LAGG is provided below.

1.9.2 Gellan gum

GG is a high molecular weight, water-soluble anionic, extracellular polysaccharide produced by the microorganism *Sphingomonas elodea* during fermentation. *Sphingomonas elodea* was previously known as *Pseudomonas elodea*. The GG polysaccharide is composed of a linear tetrasaccharide repeating unit: (1,3)-β-D-Glcp-(1,4)-β-D-GlcpA-(1,4)-β-D Glc-(1,4)-α-L-Rha-(1->) (149). Two acyl substituents, L-glyceryl and acetyl, are present in the O-3 linked glucose at the O-2 and O-6 positions,

respectively. During processing, acetylation may be lost and, depending on the degree of acetylation, GGs are typically categorised as high acyl and low acyl (Figure 1.12). The straight chain is based on repeating glucose, rhamnose and glucuronic acid units. GG is commonly used as a gelling agent, and gels formed by GG have high resistance to acid, heat and enzyme activity (150).

The United States Food and Drug Administration (FDA) has approved the use of GG as a food additive for human consumption. It was also approved by the European Community as a food additive with code E418 (151). GG is gluten free and used widely in gluten free foods to provide texture and taste to pasta, biscuits, sweets and dairy products.

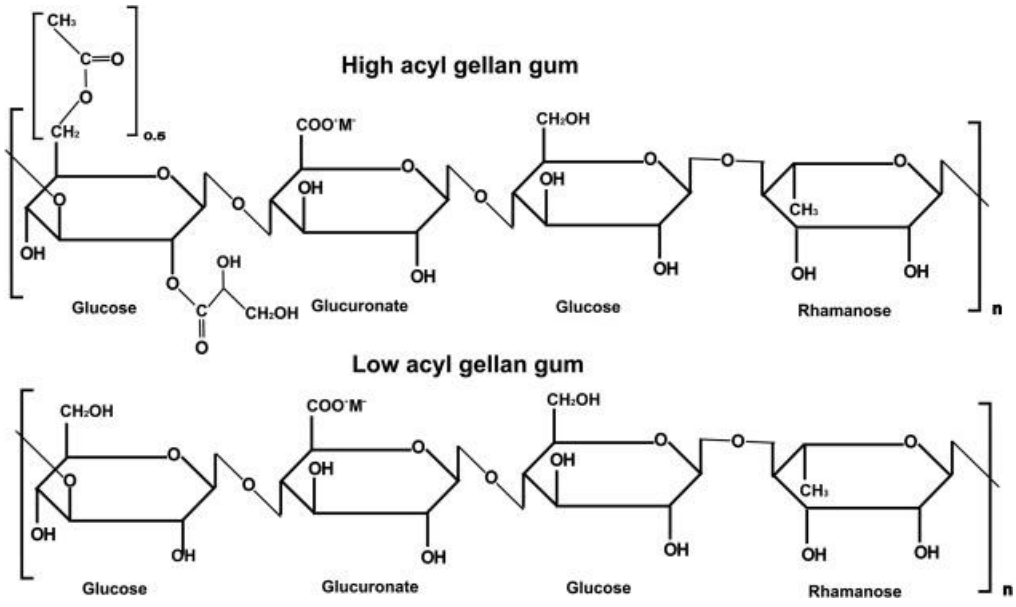


Figure 1.12 The chemical structure low acyl gellan gum (LAGG) and high acyl gellan gum (HAGG).

It is safe for people diagnosed with coeliac disease and suitable for vegetarians, kosher and Halal diets, which makes it acceptable for consumption across different populations. The GG polysaccharide increases the hardness of the rice grain (152). The addition of low acyl (LA) and high acyl (HA) GG modifies the textural properties of rice starch gel (153).

1.10 Research hypotheses and aims.

Based on the knowledge reviewed above, one can predict that adding the hydrocolloid LAGG during rice cooking could modulate starch bioavailability and that this, in turn, could modulate physiological responses to a rice meal.

Therefore, the hypotheses underpinning this work are that rice meals cooked with LAGG, compared with identical meals cooked without it, will:

1. Reduce starch digestion *in vitro.*
2. Reduce postprandial glucose blood levels and postprandial appetite *in vivo.*
3. Maintain the reduction in postprandial glucose reduction and in appetite after a sustained exposure.

Aims

1. To investigate the effect of adding LAGG to rice cooking on *in vitro* starch digestion.

2. To investigate the effect of a rice meal cooked with LAGG on the postprandial glycaemic and appetite responses in healthy humans compared with a rice meal cooked without LAGG.
3. To investigate the effect of sustained exposure to a rice meal cooked with LAGG on the postprandial glycaemic and appetite responses in healthy humans compared with a rice meal cooked without LAGG.

1.11 Thesis outline

All the work described in this thesis was conducted by the author, except where specifically credited to collaborators, at the Sir Peter Mansfield Imaging Centre (SPMIC), the School of Medicine, University of Nottingham (UoN), the Nottingham Digestive Diseases Centre (NDDC) and the National Institute for Health and Care Research (NIHR) Biomedical Research Centre (BRC) from 2018 to 2022. The research described in this thesis is original, unless otherwise stated.

The layout and content of the thesis chapters are as follows:

Chapter one: This chapter contains the thesis introduction.

Chapter two: This chapter describes general methods used in the subsequent experimental chapters.

Chapter three: This chapter describes the *in vitro* digestion studies of rice and LAGG investigating the effects on starch hydrolysis and related estimated GI.

Chapter four: This chapter describes the *in vivo* human acute intervention randomised controlled study.

Chapter five: This chapter describes the *in vivo* human sustained intervention randomised controlled study.

Chapter six: This chapter contain the overall thesis discussion, conclusions and future directions.

2 Methods

This chapter comprises brief descriptions of general experimental methods used in this work. Where appropriate, specific methods relevant to a given experimental study are reported in the corresponding thesis chapter.

2.1 Rice meals cooking methods

The rice meals for the *in vivo* studies described in Chapters 4 and 5 were cooked fresh every morning of the study according to standard operating procedures as described below.

2.1.1 Equipment

- Precision scale Ohaus Traveler TA501 (Ohaus, Parsippany, New Jersey)
- Kitchen electronic Scale (model 1036, Salter, Great Manchester, UK)
- Rice cooker (RC-8R, Cookworks from Argos, Milton Keynes, UK)
- Measuring cup (part of RC-8R rice cooker, Cookworks from Argos, Milton Keynes, UK)
- Rice Spoon (part of RC-8R rice cooker, Cookworks from Argos, Milton Keynes, UK)
- Electronic timer (model 397 SVXR Electric Timer, Salter, Great Manchester, UK)

- Disposable plastic plates, glasses and spoons (Asda, Leeds, UK)

2.1.2 Ingredients

- 2 L bottled still water for cooking (Highlands Spring, Asda, Leeds, UK)
- 330 mL bottled still water for participants (Highlands Spring, Asda, Leeds, UK)
- Thai Hom Mali fragrant jasmine rice (Green Dragon, Thailand, imported by Westmill Foods, Enfield, UK)
- Low acyl gellan gum (LAGG) KELCOGEL® F (CP Kelco, Atalanta, GA)

2.1.3 Rice control meal

The cooking and preparation of the rice control meal took 25 minutes. The standard operating procedures instructed the operator to:

1. Weigh 185 g of the dry rice using the kitchen scale
2. Weigh 356 g of room temperature still water using the kitchen scale
3. Add the rice to the water in the rice cooker bowl
4. Start the cooking function (the rice cooker trips to the ‘warm up’ function when the rice is cooked, which took approximately 20 min)

5. When cooking is complete, weigh 180 g (179.5 g rounded up to the kitchen scale precision) onto a serving plate
6. Serve with a 330 mL bottle of still water. The volunteer was given 15 min to finish all the rice meal and the water.

2.1.4 Rice plus LAGG meal

The cooking and preparation of the rice plus LAGG meal took 35 minutes. The standard operating procedures instructed the operator to:

1. Ahead of the study day, weigh an aliquot of 5.5 g of LAGG into a plastic disposable pot using the precision scale
2. Weigh 185 g of the dry rice using the kitchen scale
3. Weigh 356 g of room temperature still water using the kitchen scale and place in the rice cooker bowl
4. Add the LAGG powder to the water in the rice cooker bowl and cook for 10 min, stirring for 1 min at the beginning and for 1 min at the end to dissolve
5. Add to the rice cooker bowl 50 mL of still water to replace evaporated water
6. Add the rice to rice booker bowl and start the cooking function (the rice cooker trips to the ‘warm up’ function when the rice is cooked, which took approximately 20 min)
7. When cooking is complete, weigh 180 g (179.5 g rounded up to the kitchen scale precision) onto a serving plate
8. Serve with a 330 mL bottle of still water. The volunteer was given 15 min to finish all the rice meal and the water.

2.1.5 Observations on the cooking method

Whilst other methods of rice cooking can be used, this method was selected as it resulted in no loss of rice weight which would have occurred, if for example excess water had been added and the rice then strained. The procedures and timings detailed in the protocol were strictly followed to ensure that such factors as gelatinisation were standardised for samples both within and between the two arms of the study. Differences in outcomes could then be attributed to the addition of the GG.

2.2 Finger prick blood glucose measurement

The blood glucose levels of the participants at the fasting screening visit and during the intervention study days were measured using the finger prick technique. This measures capillary blood glucose levels. It is known that there is a small difference between blood glucose levels taken from capillary compared with venous sources (154). Venous blood is more likely to be influenced by local uptake by tissue/muscle hence it may give a less accurate reflection of whole body glucose metabolism. However the finger prick test is relatively cheap to run and much less cumbersome to set up than vein cannulation and potentially the use of a hot box to enable collection of arterialised blood. Vein cannulating also could potentially induce vagal responses and alter gastrointestinal physiology.

Briefly, the test uses a disposable sprig-loaded lance to prick the skin of the side tip of a finger and a small drop of blood is obtained for sampling using a hand-held monitor with disposable test strips.

2.2.1 Blood glucose measurement methods

Finger prick blood testing was carried out after careful hand washing, using a single-use Unistik 3 lancet (Owen Mumford, Woodstock, Oxfordshire) and an Accu-Chek Performa hand-held blood glucose meter with Accu-Chek Performa test strips (both from Roche Diabetes Care, Inc, Indianapolis, Indiana), Figure 2.1.

Figure 2.1 Participant finger prick blood test using Accu-Chek Performa reader.

As described in the Blood Glucose Monitoring Systems Accu-Chek® Performa specification (Roche diabetes, Updated Sept 2020), the AccuCheck uses an electrochemical measurement method utilising the mutant variant of the quino protein glucose dehydrogenase (Mut. Q-

GDH). The accuracy has been evaluated found to be reliable for multi-patient use (155).

The accuracy of the AccuCheck blood glucose monitors was checked against standard laboratory Yellowsprings glucose analyser 2300 (using the glucose oxidase method) in the David Greenfield Human Physiology laboratory at the UoN.

Fifteen blood samples were measured with the AccuCheck in triplicate and the mean of each blood sample measurement was compared against the corresponding measurement on the Yellowsprings meter. The test showed that the AccuCheck used for this study matched well the standard, more expensive equipment readings with linear regression R^2=0.9937 and $P < 0.0001$ aver the whole range of values of interest for this study (Figure 2.2).

2.2.2 Fasting blood glucose measurement

A finger prick measurement was carried out, in the fasting state, at the screening visit to ensure that the blood glucose of the participants was in the normal physiological range (≤ 5.4 mmol/L) before randomisation.

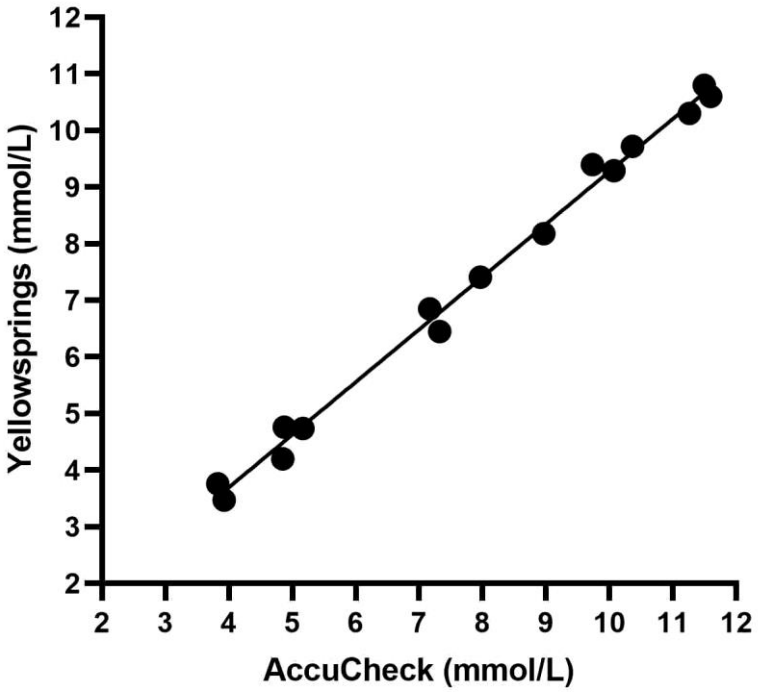


Figure 2.2 Comparison of the measurement of blood glucose carried out on 15 blood samples with the AccuCheck handheld meter against the corresponding measurements carried out using a standard laboratory laboratory Yellowsprings glucose analyser 2300. Linear regression R^2=0.9937, P < 0.0001.

2.2.3 Intervention study day blood glucose measurement

Before eating the allocated rice test meal, a fasting finger prick blood test was carried out to record the baseline fasting blood glucose level on the day. This also confirmed that the participants had fasted and that the fasting blood glucose level on the study day was within a normal physiological range.

Postprandially, fingerpick blood glucose sampling was then carried out serially until the end of the study day at the unit and time courses of the blood glucose values versus sampling time were plotted ready for analsyis.

2.2.3.1 WIGG study

For the WIGG (study acronym for White Rice and Gellan Gum) study in Chapter 4, blood glucose was sampled 15 min after meal start and every 15 minutes for 2 hours, for a total of 9 sampling points including fasting baseline. The 2 hours duration was chosen as the incremental AUC for blood glucose over 2 hours (iAUC 2h) represents the standard glucose test for assessing the glucose responses to a given glucose intake (156).

2.2.3.2 WIGG2 study

For the WIGG2 study in Chapter 5, postprandial finger prick blood glucose sampling was then carried out every 15 minutes for 3.5 hours (total of 12 sampling points including fasting baseline). This yielded the standard iAUC 2h measurements and also allowed to quantify later glucose responses, which the WIGG study highlighted as a point of interest.

2.3 Subjective appetite and symptoms measurement

2.3.1 Appetite VAS

In both *in vivo* studies paper-based 100 mm VAS were used to measure the subjective feeling of hunger, satiety, fullness, desire to eat and PFC (Figure 2.3).

These were as used previously (157, 158), with each VAS scale being anchored to an extreme sensation, (e.g. for hunger the lower anchor was ‘Not hungry at all’ and the higher anchor was ‘I have never been more hungry’). Participants were asked to make a vertical line on each of the five questions on the scale that matched how they felt at that time point.

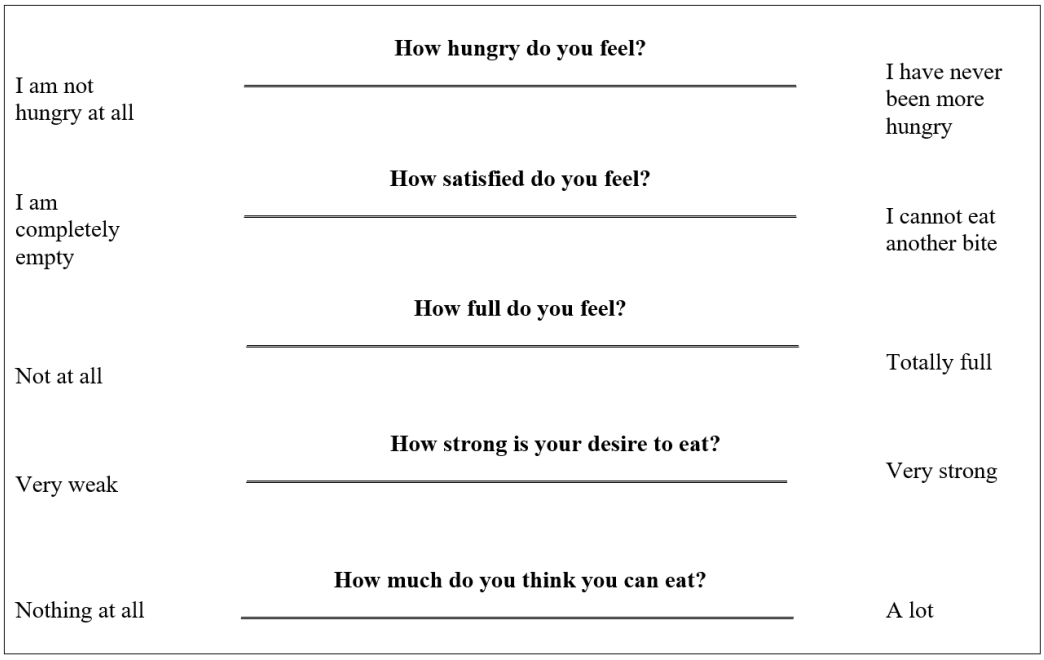


Figure 2.3 Example of the 100 mm visual analogue scales (VAS) used to measure subjective feeling of hunger, satiety, fullness, desire to eat and prospective food consumption for the *in vivo* studies.

A new VAS sheet was presented each time and removed after completion, to avoid bias from previous scores.

2.3.2 Composite appetite score

A composite appetite score was then calculated for each participant from the VAS scored for each of the 5 domains, at each time point and for each meal using the formula in Equation 2.1:

Composite appetite score = [hunger + (100 – satisfaction) + (100 – fullness) + desire to eat + prospective consumption] / 5. [2.1]

The resulting composite appetite score had values between 0 and 100. Lower values in this context indicate lower hunger, higher fullness and less desire to eat, whilst higher values indicated the opposite (53, 88).

2.3.3 Satiety Quotient

In addition to the appetite VAS scores, the use of a SQ has also been proposed by Blundell and colleagues to describe the level of satiety following consumption of a given meal (92, 94). The definition of SQ at a given time t is shown in Equation [2/2]

(Appetite rating at baseline – appetite rating at time t) / meal energy content [2.2]

The SQ can be calculated for each individual VAS for hunger, satiety, fullness, desire to eat and PFC. It can also be calculated for the composite appetite score. The SQ was calculated for all appetite scores of the *in vivo* studies in this work.

2.3.4 Observations on the appetite measurement

Visual analogue scores are a proxy measure of appetite and may not always correlate with the amount of food consumed given the complex array of factors that influence intake. However, Flint *et al.* concluded that VAS scales are reliable for appetite research and do not seem to be influenced by prior diet (88).

2.3.5 Gastrointestinal tolerance

Paper-based 100 mm VAS, as shown in Figure 2.4, were used to measure gastrointestinal symptoms of Gas/ Flatulence, Bloating, Abdominal Pain and Diarrhoea. Participants marked the point on the line that best described the severity of the symptom where 0 = represent not at all and 100 = as bad as it could be. Also, a categorical scale was used to measure the same symptoms as a second tool. The range was from 0- 3 whereby 0 = not at all ; 1 = mild (distinct but negligible) ; 2 = moderate (annoying) ; 3 = severe (disabling) (159).

Symptom category: consider if you are experiencing each symptom at the present time	**Visual Analogue Scale**: mark the point on the line that best describes how bad each symptom is 0 = 'not at all' ; 100 = 'as bad as it could be'
Gas/ Flatulence:	0 — 100
Bloating:	0 — 100
Abdominal Pain:	0 — 100
Diarrhoea:	0 — 100

Gastrointestinal tolerance Questionnaire 2

Symptom category: consider if you are experiencing each symptom at the present time	**Categorical scale**: pick **ONE** box that **best** describes how bad each symptom is **0 = not at all** ; **1 = mild** (distinct but negligible) ; **2 = moderate** (annoying) ; **3 = severe** (disabling)
Gas/ Flatulence:	☐ 0 ☐ 1 ☐ 2 ☐ 3
Bloating:	☐ 0 ☐ 1 ☐ 2 ☐ 3
Abdominal Pain:	☐ 0 ☐ 1 ☐ 2 ☐ 3
Diarrhoea:	☐ 0 ☐ 1 ☐ 2 ☐ 3

Figure 2.4 Gastrointestinal tolerance questionnaires that the participants were asked to fill at each time point. The visual analogue scale (VAS) questionnaire is shown at the top and the categorical scales are at the bottom.

2.3.6 Food records

Food diaries were given to the participants to keep a detailed record of food and beverages consumed over the prescribed study periods for the *in vivo* studies, to calculate daily energy intake. The participants were

instructed to report information on the size of the meal using household measures or by weighing the food using their own scale. They were also asked to provide information about the cooking methods and to provide brand names if appropriate. If the participants prepared a meal using several ingredients, they were asked to provide a recipe, the number of servings produced and the actual amount consumed.

An example of the food diaries and of the instruction sheet given to the participant are shown in Appendices 8.1 and 8.2.

The energy and nutrient content of the food and drink consumed was subsequently analysed using Nutritics software (Nutritics Ltd, Dublin, Ireland). The nutritional composition of non-available food items in the software were added manually using data from food labels. Where an identical food was not available, a best fit was chosen. Total energy intake for the day included the intake of rice, the *ad libitum* pasta meal and the food reported in the food diary as having been consumed over the remainder of the day.

2.3.7 Observations on food records

A variety of methods are available for the measurement of food intake which can be classified as prospective or retrospective. The 7-day weighed food intake methods is considered to be the Gold standard. All methods are however vulnerable to misreporting (160). This can be reduced by careful training, as was undertaken in this study. The degree of misreporting can be characterised by considering the ratio of recorded

intake to estimated (or measured when possible) basal metabolic rate and whether weight is stable (161, 162, 163, 164, 165). Cut offs were not calculated in this work because over the short term period of the study, weight loss would be insufficient to have enabled us to assess whether the participants were in energy balance or not.

2.3.8 Standardisation of activity and consumption prior to the study day

In order to minimise the impact of the previous day on measurement of the outcome measures on the study day, participants were required to standardise their behaviour on the previous day. Factors such as strenuous activity, consumption of alcohol and caffeine, the degree of processing of foods and composition of foods can impact on the response to subsequent meals (166, 167, 168, 169, 170)

2.4 Magnetic resonance imaging

2.4.1 MRI principles

MRI is a medical imaging technique that is able to acquire detailed images of the human body without using ionizing radiation. MRI exploits the physics properties of the protons forming the nuclei of the water hydrogen atoms. Hydrogen protons possess an electrical charge and revolve (spin) about their axis. By doing so they present a magnetic moment. When the hydrogen protons are immersed in an external,

strong magnetic field, they experience a torque which causes them to rotate (precess) around the external magnetic field. This precession happens at a frequency that depends on the external magnetic field, called the Larmor frequency. There are large numbers of hydrogen protons in a watery sample or in a human body, and collectively they present a given magnetisation vector. At this point, if a radiofrequency pulse at the Larmor frequency is sent to the hydrogen protons, this causes an excitation of the protons system and moves (tilts) the overall magnetization vector away from the external magnetic field direction. After this process, when the radiofrequency pulse is finished, the magnetisation vector will return to its equilibrium point emitting a signal that can be received by a coli (arial) and sampled. This process is the relaxation of the magnetisation vector whereby some of the energy that was provided to the hydrogen protons is released to surrounding sample/tissue, a process called spin-lattice relaxation or T1 relaxation. Conversely, when the radiofrequency pulse is turned off, the magnetic moments of the hydrogen protons are also subject to a process of dephasing in a plane perpendicular to the external magnetic field and this process is termed transverse relaxation or T2 relaxation.

Up to this point there is no spatial information attached to the signal detected from the sample or body. However, as described above the signal provided by the hydrogen protons is related to the external magnetic field. The use of three additional and orthogonal magnetic field gradients allows the operator to superimpose spatial information on the

signal that can then be mathematically reconstructed to provide a spatial MRI image of the body.

MRI has recently been increasingly used to provide novel insight on gastrointestinal physiology and on the fate of foods and drinks in the body. This can provide unique information about the intragastric appearance and emptying of the rice meals for this project.

2.4.2 MRI acquisition methods

MRI imaging was carried out using the 1.5T XDxt MRI scanner (General Electric Medical Systems, Milwakee, Wisconsin) sited at the SPMIC at the UoN (Figure 2.5).

At each time point the participants were placed in the scanner supine, feet first, with a 12-element body receiver wrapped around the abdomen. A 3-plane localizer scan was performed first to locate the organs in the body, followed by a calibration scan. This procedure took approximately 30 seconds. After this, the stomach was imaged first using an axial Half-Fourier Single-shot Turbo spin-Echo (HASTE) sequence.

This acquired 46 contiguous slices through the abdomen in a single breath-hold of 26 seconds, with a field of view (FOV) of 400 mm × 320 mm (right-left and anterior-posterior respectively). The echo time TE was 60 ms and the repetition time TR was 549.7 ms. The acquired image resolution was 1.56 mm × 1.67 mm with a slice thickness of 5.0 mm.

Figure 2.5 The 1.5T XDxt MRI scanner at the Sir Peter Mansfield Imaging Centre at the UoN.

This sequence was moderately T2 weighted therefore providing good contrast between liquids (appearing bright), tissues or hydrated foods (appearing grey), and more solid / less hydrated food components (appearing black). An example is provided in Figure 2.6 a.

Lastly, small bowel water content (SBWC) was imaged using a coronal single-shot fast spin echo (FSE) sequence. This acquired 32 contiguous slices through the abdomen in two separate breath-holds of 24 seconds, with a FOV of 400 mm × 400 mm (right-left and head-feet respectively). The TE was 326 ms and the TR was 1511.7 ms. The acquired image resolution was 1.56 mm × 3.13 mm with a slice thickness of 7.0 mm.

This sequence was heavily T2 weighted therefore showing liquids appearing very bright and other tissues and food components appearing black.

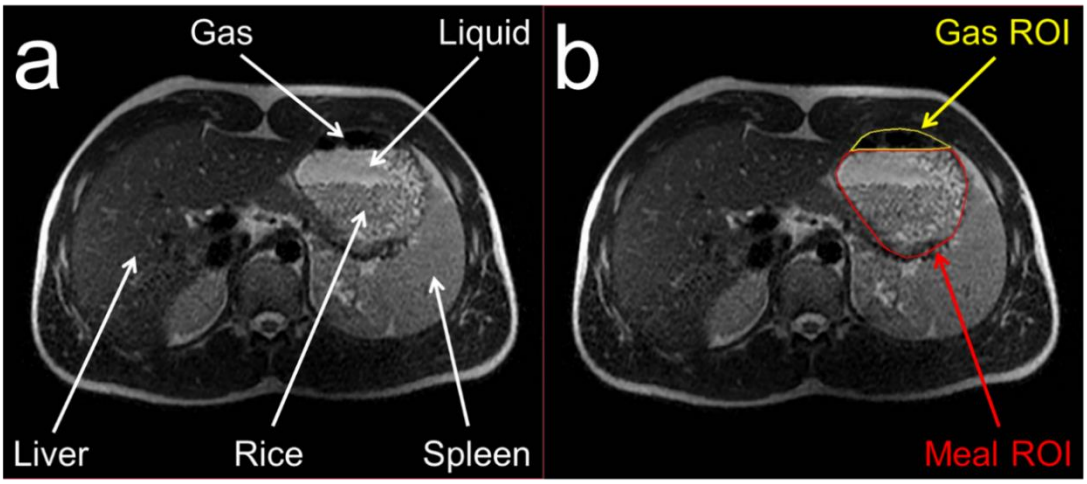


Figure 2.6 (a) Representative axial MRI image of the rice meal and water drink inside the stomach of a participant taken at T=15 minutes. (b) shows the same image but with regions of interest (ROIs) drawn over the stomach gas and stomach meal using the MIPAV software. Anatomical landmarks such as the liver and spleen are also indicated by white arrows for ease of orientation.

2.4.3 MRI image analysis methods

For the analysis of MRI, at each time point the stomach meal volume and stomach gas volume were calculated by manually drawing corresponding regions of interest (ROI, Figure 2.6 b) on the stomach images using MIPAV (Medical Image Processing, Analysis, and

Visualization software, US National Institute for Health) and summing the volumes across. Total stomach volume was then calculated as the sum of meal plus gas volume at each time point. The gastric volumes were processed by a different member of the team, Miss Katherine Riches.

SBWC was measured by a different member of the team (Prof Luca Marciani) using in-house developed software as validated and used previously (171, 172). Briefly, the SBWC assessment assumes that in the heavily T2 weighted images any bright intensity pixel with a signal intensity at and above the signal intensity of the cerebrospinal fluid within that particular image stack (i.e. as an internal normalisation) is represent freely mobile/liquid water. Based on this principle, regions of interest were manually drawn on all visible small bowel water signal and their total volume summed up by the software, yielding the freely mobile SBWC in mL at that time point.

2.4.4 Half GE time measurement

Each individual gastric volume versus time curve was fitted with both a linear and an exponential fit, and the fit with the highest R^2 was chosen to calculate the time to empty half of the gastric contents (T50%) from either a simple linear equation $V(t) = a\ t + V0$ or a simple mono-exponential equation $V(t) = V0\ e^{-a\ t}$ with no constraints on the intercepts. The average meal emptying rate (mL/min) was also calculated from the fit.

The time taken to empty 50% of the ingested contents (t1/2) has often been used to describe GE rate for the purposes of comparison.

3 Static *in vitro* digestion experiment to study the effect of adding LAGG on starch hydrolysis and estimated GI

This chapter describes the initial *in vitro* experiments carried out to investigate the effect of adding GG to rice cooking on the *in vitro* starch hydrolysis and associated estimated GI (EGI), ultimately aiming to identify the intervention for the subsequent *in vivo* studies.

3.1 Introduction

As described in Chapter 1, there are different factors that may affect the GI of white rice, such as starch type, cooking time and processing. As described by Fuwa *et al* (137), a possible way to reduce the GI of white rice through processing may be the addition of hydrocolloids. Previous work carried out by our collaborators in Birmingham and Copenhagen suggested that food hydrocolloid LAGG might be a suitable candidate with which to test this potential effect. Amongst the different white rice varieties available, jasmine rice has a particularly high GI compared with other rice varieties (173) and so it was chosen for this work as there was the greatest potential to impact on the glycaemic response.

The work described in this chapter aimed to examine the potential effect of adding different amounts of LAGG to jasmine rice during cooking on glucose bio-accessibility and EGI through an *in vitro* digestion model.

This would enable the optimal intervention to be selected for the subsequent *in vivo* studies.

The work also aimed to label LAGG with a fluorescent molecule and to use fluorescent microscopy to investigate whether LAGG physically coated the rice grains during cooking and if it penetrated below the surface of the grain. This would provide further insight into the model of action of the hydrocolloid in reducing the GI of white rice.

3.2 Materials and methods

3.2.1 Materials

LAGG (KELCOGEL® F) was obtained from CP Kelco (Atalanta, GA). Thai Hom Mali fragrant jasmine rice (Green Dragon, Thailand, imported by Westmill Foods, Enfield, UK) was purchased from a local superstore (Medina Continental Foodstore, Nottingham, UK).

Salivary alpha-amylase, pancreatic alpha-amylase, pancreatin, bile salt, pepsin enzyme, assay 4-hydroxybenzhydrazide (PAHBAH), maltose standard, stop solution (Na_2CO_3) and all other reagents were of analytical grade and purchased from Sigma–Aldrich (Merck Life Science, Gillingham, UK).

3.2.2 Sample preparation

Ten grams of raw jasmine rice were cooked in 15 g of water at 95°C for 20 minutes with the addition of different amounts of LAGG, expressed as

a percentage of LAGG weight over raw dry rice weight. Samples were initially cooked with a range of LAGG amounts to determine the sensible working range before the water phase become too thick to undertake the necessary subsequent processing. Sample A was the control cooked with no added LAGG. Samples B, C and D were cooked with 1%, 2% and 3% added LAGG (w/w of dried rice), respectively. After the cooking was completed, the rice was mixed gently with a spoon. Samples were prepared fresh just before studying their digestion *in vitro*, and the cooking and digestion procedures were repeated in triplicate for each sample.

3.2.3 *In vitro* digestion

For *in vitro* digestion, the static INFOGEST protocol was followed with some modifications (174, 175). Briefly, 5 g of the cooked rice was transferred into 50 mL conical tubes. For the oral phase no mastication step was used. Five mL of simulated salivary fluid (SSF) containing 75 u/mL human salivary amylase (Sigma–Aldrich) was added to each tube, vortex mixed (Fisher Brand, UK), and left to stand for 2 minutes. The gastric phase was then initiated by adding 10 mL of simulated gastric fluid (SGF) containing 288 mg of pepsin (Sigma–Aldrich) to each tube. The samples were vortex mixed and incubated at 37°C for 30 min at pH 1.5. For the intestinal phase, 20 mL of simulated intestinal fluid (SIF) containing 4 mL of pancreatic a-amylase (Sigma–Aldrich) were added to each tube, and the tubes were vortex mixed and incubated at 37°C for

120 min at pH 6. Examples of key laboratory equipment used are shown in Figure 3.1.

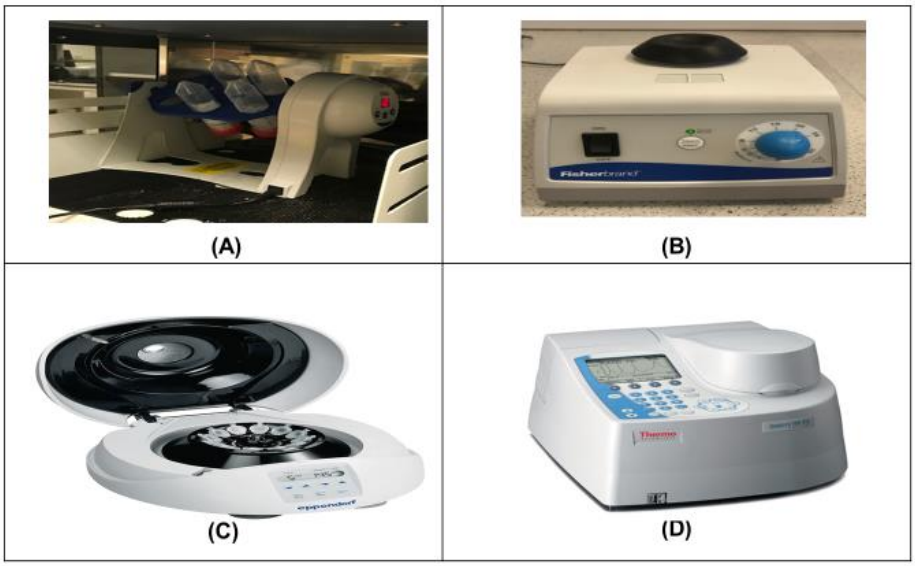


Figure 3.1 (A) Rotator,ThermoScientific,USA - Incubator,Sturat,UK. (B) Vortex Mixer,Fisher, UK. (C) Microcentrifuge, Eppendort, Germany. (D) Genesys 10 Vis spectrophotometer, Thermo Fisher Scientific, Waltham, MA.

3.2.4 Sampling and determination of starch hydrolysis curve

At 2.5, 5, 10, 15, 20, 30, 45, 60, 90 and 120 min of intestinal digestion (Figure 3.2), a 200 µL aliquot was transferred from the digestion tube to a 1.5 mL test tube containing 200 µL of stop solution (0.3 M Na_2CO_3 aimed to stop enzyme activity by increasing the pH above the activity

range) and vortex mixed to inactivate the α-amylase. Intestinal digestion started at time 0 with the addition of SIF containing pancreatic enzymes.

1	2	3	4	5	6	7	8	9	10
2.5 min	5 min	10 min	15 min	20 min	30 min	45 min	60 min	90 min	120 min

Figure 3.2 Time points of *in vitro* digestion sampling.

There was necessarily a delay from adding the intestinal digestive fluids to taking the first sample (i.e., time required to mix the sample with the fluids and measure the pH) and therefore the first sampling time was standardised to 2.5 min for all samples, for consistency. Also, care was taken to avoid taking large particles from the sample in the aliquot. Starch hydrolysis was measured as released reducing sugars using a 4-hydroxybenzhydrazide (PAHBAH) assay (176).

The samples were first centrifuged for 5 minutes, then 20 µl of the supernatant was transferred to a new tube and diluted in water (50:1). Next, 100 µL of the diluted supernatant was transferred into a new tube, 1 mL of freshly prepared PAHBAH solution was added (250 mg PAHBAH dissolved in 4.75 mL of 0.5M HCl, then mixed with 45 mL of 0.5M NaOH) and the tube was placed into a 95°C water bath for 5 min. The samples were then allowed to cool to room temperature before transferring to cuvettes. Absorbance at a wavelength of 405 nm measured using a

spectrophotometer (Genesys 10 Vis, Thermo Fisher Scientific, Waltham, MA) was then compared with the maltose standard curve. The rate of starch digestion was expressed as the percentage of total starch hydrolysed at different times with 100% indicating hydrolysis of the total starch content of the investigated rice portion. The percentage of starch hydrolysis of the study samples was calculated based on the method of Goni (177).

A similar digestion experiment was carried out using white bread as a sample. The hydrolysis index (HI) was obtained by dividing the area under the hydrolysis curve of each rice sample by the corresponding area of the reference sample (white bread). The EGI was then calculated using Equation 2.1, as suggested by Goni *et al.* (177):

$$GI = 39.71 + (0.549\ HI) \qquad [2.1]$$

3.2.5 Fluorescent microscopy

LAGG was labelled using 5-(4,6-dichlorotriazinyl) aminofluorescein (5-DTAF). 5-DTAF is a reactive dye usually used to stain proteins (amine and thiol groups) and has an absorption/emission maxima of 492/516 nm. The 5-DTAF label can bind strongly to the LAGG and therefore provide a unique fluorescent probe to demonstrate if the rice grain is coated by LAGG during cooking and/or if LAGG is able to penetrate below the grain surface. The staining protocol was developed by Dr Gleb Yakubov's group in the Division of Food Sciences, Nutrition and Dietetics, Sutton Bonington, UoN, UK. They also carried out the staining

and fluorescent microscopy study. The details of the complex staining protocol are beyond the scope of this chapter and will only be summarised briefly below. A stock solution of 40 mg of 5-DTAF in 4.038 mL of DMSO was prepared. 0.2 mM of 5-DTAF was added to 10 mg/mL of LAGG sample at room temperature. Ten mM Na_2SO_4 was slowly added to this. The pH was then raised to 10 using NaOH at room temperature and after two hours the reaction was quenched using 1:2 ratio ethanol/sodium acetate buffer mixture. After this the unbound 5-DTAF was washed using ethanol:sodium acetate solution using a Buchner funnel with a 0.45 μm PTFE membrane under vacuum until the filtrate appeared clear. The fluorescently labelled LAGG was then dehydrated using ethanol and dried in the vacuum overnight, ready to use.

After this, the same cooking protocol described in paragraph 2.2.2 was followed using jasmine rice with 3% weight / dried rice weight unlabelled LAGG (control) or labelled LAGG. The cooked rice was then cut using a blade and underwent fluorescent microscopy in the Food Sciences Building laboratory facilities, Sutton Bonington, UK.

3.2.6 Statistical analysis

Data were collected in triplicate and it was assumed that that data were not normally distributed. Therefore, data are reported as medians with an interquartile range or 95% confidence intervals. A two-way ANOVA analysis was used to assess the main effects of percentage LAGG and

a time on the *in vitro* starch hydrolysis. A non-parametric Kruskal-Wallis test, followed by Dunn's *post-hoc* multiple comparisons test, was used to evaluate differences between the estimated GI values. The data were analysed using GraphPad Prism for Windows version 9.2.0 (GraphPad Software, San Diego, California).

3.3 Results

Initial pilot work to find the LAGG dose range for the work used 1%, 2%, 3% and 4% LAGG (w/w of dry rice). This had demonstrated that above 3% w/w of dry rice the water phase was too thick for the subsequent processing, therefore 3% LAGG was chosen as the upper limit for the work described below.

3.3.1 *In vitro* starch hydrolysis

The kinetics of *in vitro* starch hydrolysis for cooked jasmine rice and cooked rice with different percentages of LAGG are shown in Figure 3.3. The degree of *in vitro* starch hydrolysis in 1%, 2% and 3% LAGG rice were lower than that of the rice sample without LAGG at all times, and the effect of starch hydrolysis was dose dependent on the LAGG percentages. At 120 min, *in vitro* starch hydrolysis for 1%, 2% and 3% LAGG jasmine rice was reduced by 35%, 40% and 50%, respectively, compared with control rice. The profile of *in vitro* starch hydrolysis (Figure 3.3) showed a gradual increment in starch hydrolysis in all tested rice

samples, with an apparent dose-response reduction as LAGG percentage increased.

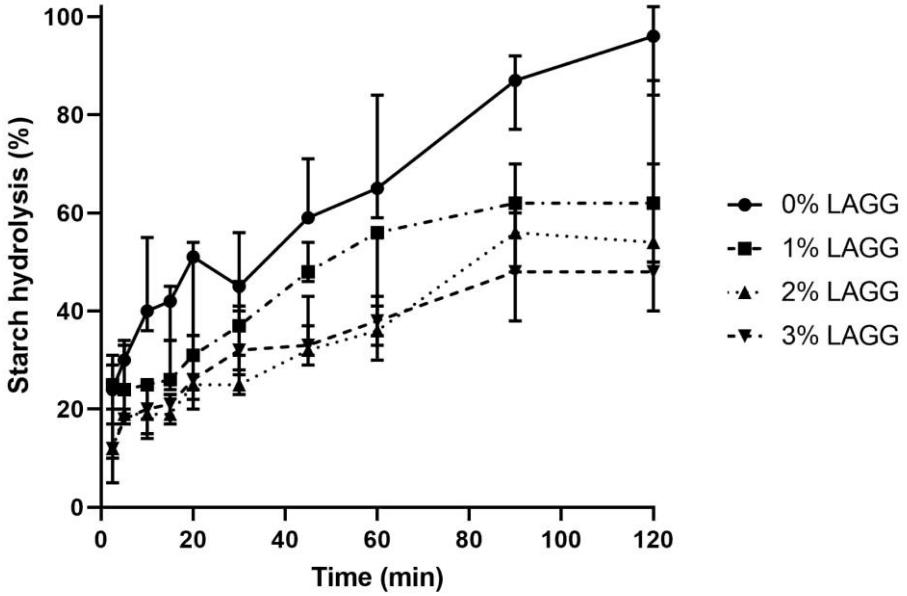


Figure 3.3 *In vitro* starch hydrolysis (%) over time for samples of jasmine rice cooked with increasing amounts of LAGG. The vertical axis indicates the hydrolysed % of the total starch content of the investigated rice portion. Each data point is from triplicate measures and data are shown as median ± 95% confidence intervals.

These data are in triplicate which made it difficult to test for normality of the data. Assuming normality, a two-way ANOVA analysis showed a significant main effect of percentage gellan (P = 0.0133) and a significant main effect of time (P = 0.0126) however there was not a significant interaction (P = 0.2389).

3.3.2 *In vitro* estimated GI

The addition of LAGG affected rice starch hydrolysis in the static *in vitro* model of digestion, with an incremental effect. The effect of LAGG addition on the estimated GI values of cooked rice is shown in Figure 3.4, whereby the medians vary significantly with % LAGG, Kruskal-Wallis test $P = 0.0014$. LAGG significantly reduced the estimated median GI value by 27%, from 94 for the control 0% LAGG, to 69 for the 3% LAGG samples ($P < 0.05$ *post-hoc* Dunn's multiple comparisons test difference from control sample 0% LAGG).

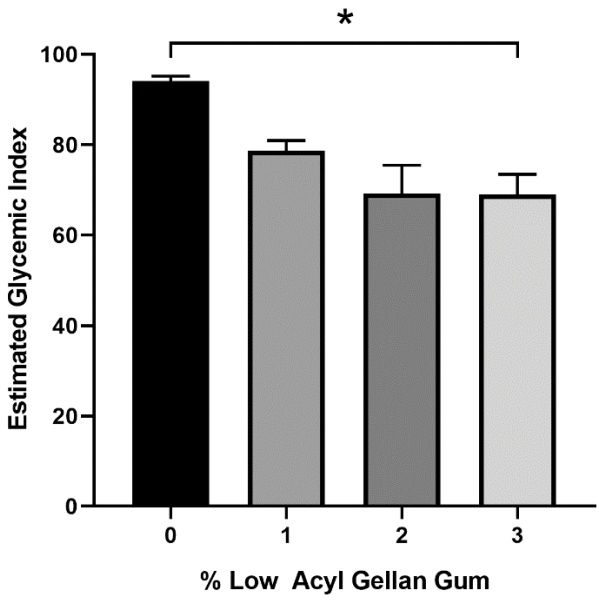


Figure 3.4 Estimated glycaemic index of jasmine rice cooked with increasing amounts of LAGG, as determined by the *in vitro* static digestion model. The data are shown as medians and IQR of the triplicate samples. The medians varied significantly with % LAGG, Kruskal-Wallis test $P = 0.0014$. *$P < 0.05$ *post-hoc* Dunn's multiple comparisons test difference from control sample 0% LAGG.

3.3.3 Fluorescent microscopy

Example results of fluorescent microscopy images of cross sections of jasmine rice grains cooked with and without the 5-DTAF labelled GG are shown in Figure 3.5. Jasmine rice with 3% weight / dried rice weight unlabelled LAGG (control) or labelled LAGG was used. On panel (b) the green fluorescence arising from the labelled LAGG is clearly visible. The images confirm that using the current cooking protocol the LAGG did indeed coat all the outer surface of the rice grains and also penetrated below the surface towards the middle of the grains. In the control study (a) no fluorescent signal was visible as expected.

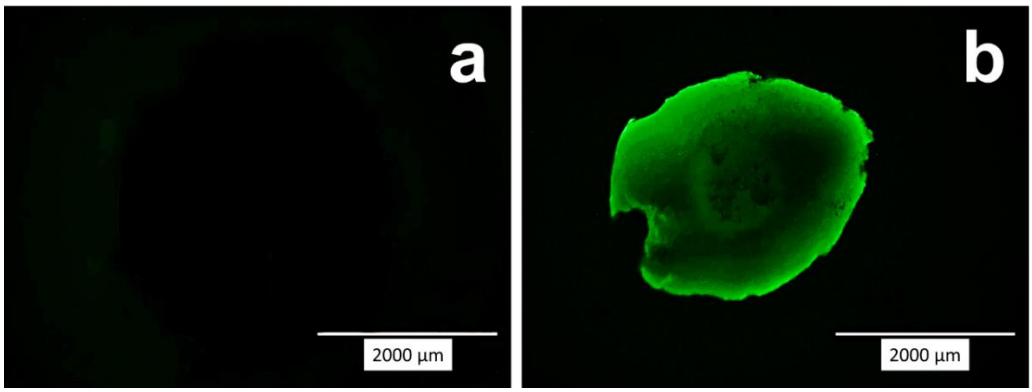


Figure 3.5 Fluorescence microscopy images of: (a) a cross section of a grain of jasmine rice cooked with 3% (w/w of dried rice) unlabelled low acyl gellan gum (LAGG) as control. (b) a cross section of a grain of jasmine rice cooked with 3% (w/w of dried rice) LAGG that was labelled with fluorescent dye 5-(4,6-dichlorotriazinyl) aminofluorescein (5-DTAF).

3.4 Discussion

An *in vitro* starch hydrolysis method, simulating the *in vivo* process of carbohydrate digestion, was utilised in this study to compare the starch digestibility profiles of rice samples with different amounts of LAGG added to the cooking process, and to estimate their associated *in vitro* GI. This was done in order to characterise and predict the metabolic glycaemic response to food (177) with a view to selecting the interventions for the *in vivo* study.

This digestion study investigated the effect of adding food hydrocolloid LAGG at 1%, 2% and 3% to white rice (jasmine) on starch hydrolysis and the EGI of white rice. The common 2 hours digestion time was used for this *in vitro* evaluation as also mentioned in the original INFOGEST method (174, 175). This length has been used previously in the literature, for example in the classification of different types of starch, rapidly digestible, slowly digestible, and RS (178). In the Figure 3.3 it can be seen that some hydrolysis is still ongoing at the 2 hours mark. In future studies the *in vitro* digestion model could be run for longer. The 2 hours length mirrors that used in the *in vivo* acute studies described in Chapter 4. In the chronic feeding study described in Chapter 5, the time window used for blood glucose sampling *in vivo* was longer, at 3.5 hours, as this provided an opportunity to examine changes in blood glucose in response to a more complete GE of the rice meal.

The results obtained indicated clearly that the addition of LAGG to jasmine rice reduced the rate and extent of starch hydrolysis over the two hour period and the associated EGI at 1%, 2% and 3% compared with

the control cooked jasmine sample. The results of the present study are in agreement with a previous study carried out by our collaborators in Birmingham and Copenhagen to investigate the effect of adding 1% LAGG to jasmine rice during cooking (148). The highest effect of starch hydrolysis inhibition was observed here at the highest amount of 3% LAGG. Attempts to increase the LAGG percentage beyond 3% showed that the cooking water started to become too thick before addition of the rice, and therefore 3% was taken as the optimal dose for the development of the next stages of this work.

Having that the addition of LAGG during cooking reduced the EGI of jasmine rice, it was necessary to establish whether a possible mechanism might be that the LAGG coated the rice grains. GG is one of the readily available food hydrocolloids. However, research regarding the effect of GG on starch hydrolysis and GI is poorly explored in the literature compared with other food hydrocolloids.

The 5–DTAF labelling experiment was designed for this purpose in collaboration with colleagues from Dr Gleb Yakubov's group at the Food Sciences Laboratory at the UoN. 5-DTAF can only bind to GG through the chemical labelling process. The labelled gum is then added to the cooking process. Therefore, the green fluorescence signal detected in Figure 3.5 showed LAGG distribution on the rice grain, not only on the surface but also with good penetration inside the grain.

This indicates a possible mechanisms for how LAGG reduces starch hydrolysis and EGI. LAGG does coat the surface of the rice grain, thus it

may act as a barrier for enzymatic access and it may also restrict the leakage of amylose during gelatinisation of the starch granule. Norton et al. suggested that LAGG can resist the acidic environment in the stomach well (179). Modifying the rice cooking process by adding LAGG may create an outside and also inside barrier to enzymatic action around the rice grain.

The addition of LAGG and HAGG has been reported to modify the textural properties of rice (153). A study by Huang et al. reported that GG increased the hardness of the tested rice (145). One can hypothesise that this may strengthen the grain texture although grain compression strength was not tested here.

It could also possibly strengthen aggregation of the individual rice grains during bolus formation after swallowing, further reducing surface exposure to enzymes during digestion. This is something that *in vivo* imaging experiments could potentially detect.

In summary, modifying the rice cooking process with LAGG may create an outside and also inside barrier to enzymatic action, may strengthen the grain texture; it could also possibly strengthen aggregation of the individual rice grains during bolus formation after swallowing, further reducing surface exposure during digestion. This is something that *in vivo* imaging experiments could potentially detect. Other mechanisms at play would involve competitive binding of amylase to the LAGG. These mechanisms could also potentially be amplified by further gelation and increase in gel strength of the LAGG in the acidic stomach environment.

Through its gelling action, LAGG may therefore slow GE and reduce further the rate of starch digestion.

GG is considered safe for dietary consumption. The United States FDA has approved its use as a food additive (151). It was also approved by the European Community as a food additive with code E-418. Moreover, GG is gluten free and is broadly utilised in gluten free foods to improve the texture and taste of pasta, biscuits, sweets and dairy products. It is harmless for people diagnosed with coeliac disease and is suitable for vegetarians, kosher and Halal diets, which means it is suitable for consumption across different populations.

The use of an *in vitro* digestion model was very useful for testing the initial hypothesis that adding LAGG to the cooking process of the rice would reduce starch digestion *in vitro*. *In vitro* studies have many benefits in terms of timescale, replication and cost. However, the human digestive system is complex, and *in vitro* models can only represent the complexity of the human digestive system to a certain extent (174, 175).

More specifically, the INFOGEST *in vitro* digestion model used here had some limitations (180). The model was based on a static method, and as such it does not account for dynamic changes that would occur during normal *in vivo* digestion, including pH changes and feedback mechanisms. Despite this, several studies have shown good correlations between the static INFOGEST digestion model and *in vivo* data. The method has been developed with reference to healthy individuals, as planned for the work here, and the rice meals are low on lipids,

overcoming a known limitation of the INFOGEST model with fatty acid digestion. A semi-dynamic INFOGEST method has been developed too (181), but it is quite complex to set up and for the purpose of this study it was deemed that the static model was sufficient to screen and select materials for the planned *in vivo* studies.

This study was carried out on a particular variety of rice, jasmine white rice , and its applicability to different varieties of white rice remains to be investigated. The effect of hydrocolloids on the properties and digestibility of white rice would probably depend on many factors, including the surface properties of the rice. One can hypothesise that for highly refined white rice grains the effect shown here would be relatively similar and transferable irrespectively of the rice variety.

A limitation of this work was the lack of a mastication step at the beginning of the *in vitro* digestion process. Mastication and the concurrent mixing with saliva in the mouth can of course affect digestibility. However, the main aim of this work was to compare rice samples for starch digestibility with and without the hydrocolloid under simple and similar digestion conditions. Another limitation of this *in vitro* work was that no negative control (i.e. a sample with no rice) was used. This would have confirmed that a sample without starch provides marginal starch hydrolysis products in the analysis. Whilst expected, confirming this experimentally would have added strength to the study design and results. The main interest of the *in vitro* experiments was nevertheless to compare rice without hydrocolloids to rice with hydrocolloids. This comparison was possible and meaningful without the

use of a negative control. In future work the inclusion of a a negative control should however be considered.

White rice was chosen as it is highly preferred by a large segment of the population, it has high GI, and its consumption has been associated with adverse health effects in regions where rice is a staple food. Brown rice was not considered here because it already has a low GI and therefore the intervention potentially would be less effective. Hydrocolloids, including GG, could interact with brown rice in a different manner compared to white rice, as the surface properties of the brown rice grains will be different.

3.5 Conclusion

Based on the results of the present study, *in vitro* starch hydrolysis of rice samples was significantly reduced by the addition of LAGG to rice through cooking. The effect was dose dependent. The highest dose of LAGG (3%) showed the highest reduction in starch hydrolysis and, in turn, the lower EGI. This could be beneficial in reducing postprandial glucose levels *in vivo*. This hypothesis therefore needed to be tested in a human clinical trial, which is described in the following chapter of this thesis.

4 The effects of LAGG on the glycaemic, gastrointestinal and appetitive responses to a white rice meal

Building on the *in vitro* study, the work described in this chapter aims to evaluate the effects of adding LAGG to jasmine rice processing on acute postprandial blood glucose response in healthy adult participants.

4.1 Introduction

Short-term studies have demonstrated that foods with low GI tend to increase satiety and lower energy intake (182). Populations that eat large amounts of high GI foods are thought to be at increased risk of developing T2DM and CVD (103). White jasmine rice is low in dietary fibre and has a high GI, as demonstrated by the marked postprandial blood glucose response. High consumption of white rice has been associated with increased risk of developing T2DM, which has been attributed to the high glycaemic response to the rice (106, 183, 184).

The work completed in Chapter 3 suggested that the addition of LAGG to the cooking process of white jasmine rice can reduce the glycaemic response of the rice in an *in vitro* digestion model. This suggests that LAGG could be used to reduce the glycaemic response of rice *in vivo*. This hypothesis is also supported by evidence showing that other

hydrocolloids can reduce the glycaemic response to usually high GI foods (185). There is only one report in the literature exploring the concept of lowering the GI of rice by using hydrocolloids during food processing. In that study, different combinations were considered of xanthan gum (a common polysaccharide hydrocolloid, similar to LAGG, in that it is of bacterial origin) and Japanese 'Koshiibuki' polished rice and how it may affect blood glucose (137). They found that the addition of more than 1% xanthan gum to the cooking of rice decreased postprandial blood glucose levels 15 and 30 min after consumption compared with rice cooked alone. However, the effect of adding LAGG to rice during cooking still remains to be investigated.

The main aim of this two-arm, randomised, controlled, cross-over study was to explore the impact on glycaemic response of adding LAGG to jasmine rice during the cooking process. The hypothesis to be tested was that LAGG will reduce the glycaemic response to white rice *in vivo* in healthy humans. The primary objective was thus to test the impact of LAGG on the primary outcome measure, the incremental AUC for blood glucose measured using the finger prick technique. It was also proposed that the addition of LAGG to a rice meal would affect appetite and gastrointestinal response. Exploring the impact of adding LAGG to jasmine on appetite and gastrointestinal responses were thus secondary objectives and the following secondary outcome measures were considered following consumption of a rice test meal: subjective appetite response measured using visual analogue scores and objective food intake at a pasta meal, reduction in gastric volume and SBWC. Following

the consumption of the pasta meal, subjective appetite responses and consumption over the remainder of the day also were measured. Estimation of total energy intake over the day was then estimated by summation.

4.2 Methods

4.2.1 Study design, Ethics and randomisation

This study was a randomised, controlled, cross-over trial with two arms The intervention consisted of a single meal of jasmine rice cooked with LAGG. The control consisted of a single meal of jasmine rice cooked without LAGG. Participants attended the laboratory for two study visits of approximately 2.5 hours, one week apart when they consumed either the intervention meal or the control meal.

The study was approved by the UoN Faculty of Medical and Health Sciences Research Ethics Committee (Ethics approval number 470-2001) and it was registered on clinicaltrials.gov with Identifier NCT05080400. The study took place at the SPMIC at the UoN between July 2021 and November 2021. Informed written consent (Appendix 8.3) was obtained from each participant before the trail. A case report form (CRF) was completed for each participant according to good clinical practice.

Randomisation was carried out by the study investigator using a simple Latin square design in order to minimise order effects. Allocation to the intervention was carried out following the Latin square design with participants allocated in a consecutive sequence matching prospective

recruitment numbers to avoid allocation bias. There was no selection bias and participants were recruited consecutively if they matched the eligibility criteria at screening.

Participants were requested to consume the same meal of their choice on the evening prior to each study visit, and to consume their habitual diet during the week between study visits.

The initial design was single-blind with the participants kept blind to the rice intervention cooking process. However, after data collection the data sets were further blinded to the operator by a member of staff not involved with the analysis and the blinding code was broken only after the analysis.

4.2.2 Eligibility

The inclusion criteria were:

- Aged between 18 and 65 years old.
- Able to give informed consent.
- BMI ≥18.5 and ≤24.9 kg/m²
- Apparently healthy: no medical conditions or previous gastrointestinal surgery which could affect study measurements assessed by the General health checklist (Appendix 8.4), reviewed by the study investigator.

Exclusion criteria included:

- Fasting screening blood glucose greater than 5.4 mmol/L measured by finger prick.
- Restrained eating behaviour as determined by SCOFF screening questionnaires (186) (shown in Appendix 8.5)
- Use of medication which interferes with study measurements
- Participation in another nutritional or biomedical trial 3 months before the screening appointment or during the study
- Dislike of the products served as the dietary test treatments.
- Any allergy or food intolerance to the test treatments
- Not suitable for MRI scanning (e.g., presence of metal implants, infusion pumps, and pacemakers) as assessed by standard MRI safety questionnaire (Appendix 8.6).
- Pregnancy
- Inability to lie flat as would be required for the MRI.

4.2.3 Recruitment

Participants were recruited via posters placed around the UoN. Those expressing an interest were sent the participant information sheet (PIS) to read and if interested, they were then asked to attend an appointment of approximately 30 minutes at the study unit when informed written consent was obtained and then screening was undertaken to determine if they were eligible for the study. Potential participants were asked to fast for at least 11 to 12 hours prior to the appointment. Water was allowed until 1 hour before the appointment. Screening included a finger prick blood sample to test for fasting blood glucose level (for details of

the method see Section 2.2.2). Anthropometric data were also collected, including weight and height to calculate the BMI as defined in Section 1.1. The participants filled in a Healthy volunteer's written consent form (Appendix 8.3), a general health checklist (Appendix 8.4), a SCOFF screening questionnaire (186) (Appendix 8.5) and an MRI safety questionnaire (Appendix 8.6). Twenty-one participants were screened, of whom 13 were randomised and 12 completed the study as shown by the study Consolidated standard of reporting clinical trials (CONSORT) flow diagram (Figure 4.1).

4.2.1 Study protocol and procedures

The participants arrived at 09:00 hours having fasted since 22:00 hours the previous evening and having avoided alcohol, strenuous exercise, and caffeine for the 24 hours prior. Participants filled out a study day eligibility check questionnaire (Appendix 8.7) and filled in again a MRI safety form (Appendix 8.6) on each study day to ensure they had adhered to the instructions previously given and were safe to go in the MRI scanner.

The trial design is shown in Figure 4.2 and a schematic diagram of the measurement times of the study day protocol is shown in Figure 4.3.

Before the rice test meal, termed as time T=0 throughout, a fasting finger prick blood test was carried out using the methods and equipment as described in Section 2.2.2.

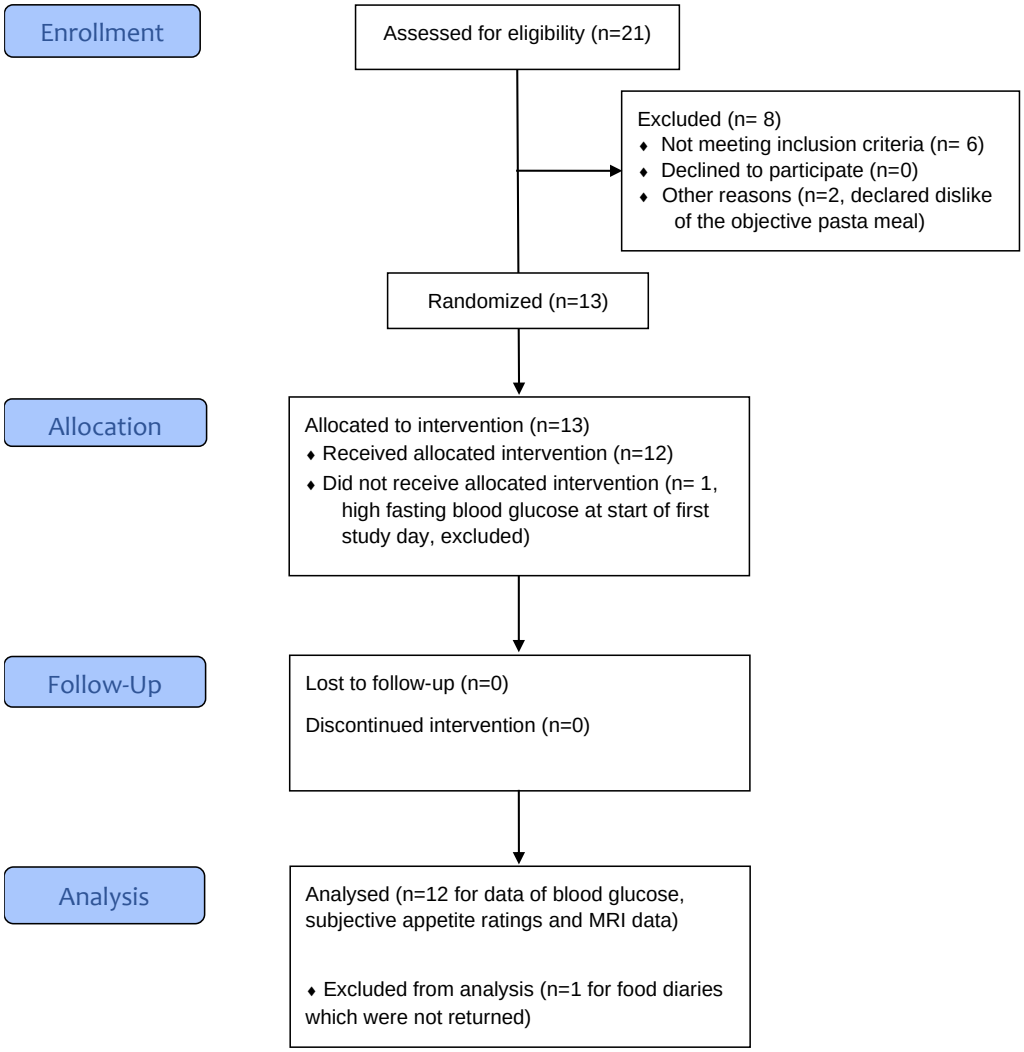


Figure 4.1 CONSORT 2010 Flow Diagram (187).

This test provided the baseline fasting blood glucose level and confirmed that the fasting blood glucose level on the study day was within the eligibility criteria. The participants then filled in the baseline VAS described in Section 2.3. They were then positioned supine in the MRI scanner and underwent a MRI scan of the abdomen. This provided

baseline images of the stomach which also confirmed that the participants had fasted as requested so the stomach did not contain food at the start of the study.

The participants were then asked to sit at a table in a quiet room with no mobile phones or distractions and they were provided with the rice meal immediately after it had been cooked. They were asked to consume the rice test meal within 15 min. The gum was tasteless under the investigated conditions. Both rice meals were presented on a white paper plate in exactly the same way to ensure blinding. An identical white plastic spoon was provided in both cases. The rice was provided with 330 mL of room temperature still water in a bottle and the participants were asked to consume all of the water with their meal.

Postprandially, fingerpick blood glucose sampling was then carried out 15 min after the meal start and every 15 minutes for 2 hours (total of 9 sampling points including fasting baseline). MRI images of the abdomen were also taken after feeding (T=15 min) and subsequently at 30-minute intervals for 2 hours (6 MRI sampling points in total). The finger-prick, followed by the VAS for appetite were carried out immediately before the MRI scan. The MRI procedures took only a few minutes and the participants spent most of the time sitting up in a quiet room adjacent to the MRI scanner, undertaking a quiet activity such as reading or working on a laptop. When this was all completed, an *ad libitum* pasta lunch was provided approximately 15 min after the final MRI scan, followed by one last VAS questionnaire. The participants were then instructed to keep food diaries (Appendices 8.1 and 8.2) which they were provided with for

the rest of the day, to calculate the total energy intake for the day. On the first visit, the second visit date was confirmed following a washout period of 7 days. They were then discharged.

At the end of their last visit, the participants were asked 3 yes/no standard questions: if the study was acceptable, if the meals were acceptable and if they perceived a difference between the two meals.

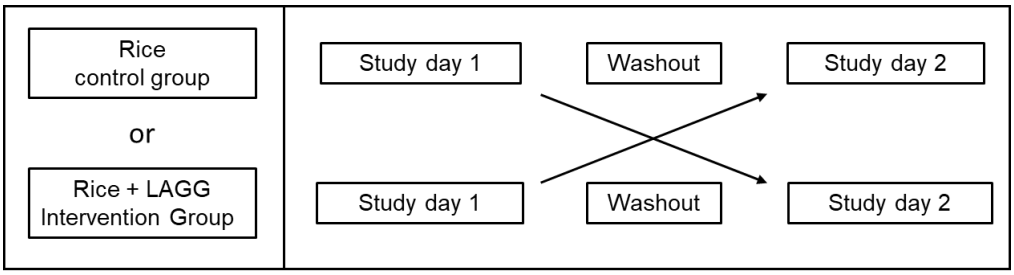


Figure 4.2 Trial design for the study. The washout period was 7 days.

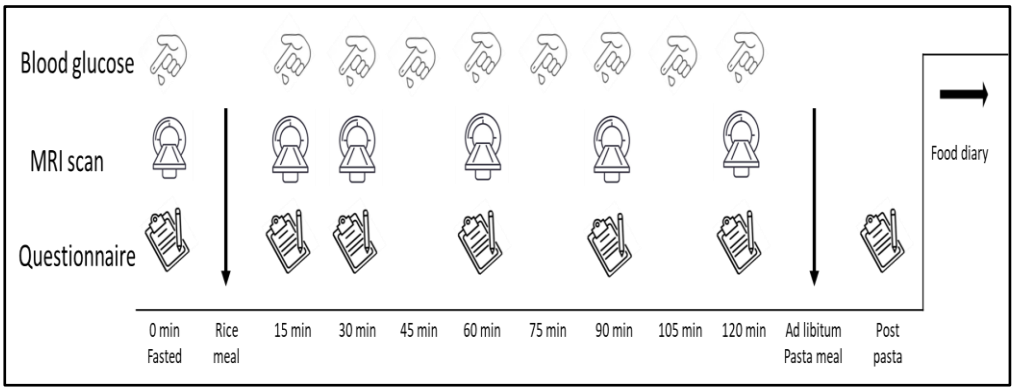


Figure 4.3 Schematic diagram of the events during the human MRI study day. The questionnaires consisted of VAS (Section 2.3.1).

4.2.2 Rice test meals

The study comprised two rice test meals based on jasmine rice (as described in Section 2.1) cooked in water. The control rice meal was prepared using 185g of raw jasmine rice placed with 365mL of still bottled water (ASDA, UK) in a 1.5 litre rice cooker (Cookworks™, Argos, UK). The rice cooker took 20 minutes to cook the rice before switching off automatically, at which point all the cooking water had been absorbed by the rice. After this 180g of the cooked rice was weighed and served in a disposable dish with a disposable spoon and a 330mL bottle of still water (Highland Spring, UK). The standard operating procedures for cooking the rice meals are reported in Section 2.1.

For the 3% LAGG rice meal preparation, firstly 5.5 g of LAGG (equivalent to a 3% w/w of dried rice) were dispersed in 365 mL water, with the rice cooker turned on, for 10 minutes. The water/gellan mixture was then stirred and an additional 50mL of water was added to replace water lost as previously determined by a test of water weight loss on cooking. The aliquot of 185g of raw rice was then added and cooked in the same way as above and fed immediately after cooking.

The two rice meals looked identical, as seen in Figure 4.4. The cooked meal portion corresponded to 50g of available carbohydrates (185), which is the standard used to test the GI of foods, and the meals provided 232 kcal each (isoenergetic) with the energy content of the GG considered to be zero kcal/g as recommended (188).

For the purpose of participants blinding, both rice meals were presented in the same way, to ensure that the participants remained blinded to the intervention. The gum is tasteless and the final texture of the two meals was only marginally different. The participants were asked at the end of the study a set question about study acceptability and difference between meals.

Figure 4.4 Appearance of the rice meals after cooking: (A) rice control and (B) rice with LAGG.

4.2.3 Outcomes

4.2.3.1 Glycaemic response

Finger prick blood testing was carried out as described in the methods Chapter at Section 2.2.

4.2.3.2 Subjective appetite responses

VAS were used to measure the subjective feeling of hunger, satiety, fullness, desire to eat and PFC as described in the methods Chapter (Section 2.3.1). A composite appetite score was subsequently calculated from the five VAS scores (Section 2.3.2).

4.2.3.3 *Ad libitum* meal

An *ad libitum* meal was served approximately 15 min after all the glucose testing and MRI procedures were completed, as a lunch test, to assess *ad libitum* food intake. It consisted of a tomato and mozzarella pasta bake purchased from the chiller cabinet of a supermarket (Tesco, UK, Figure 4.5).

Three packs (450 g each) of pasta bake were heated in a 900 W microwave at full power for 10 min and stirred well at the end. The nutritional value of the pasta per 100 g was 109 kcal, comprising 16.4 g of carbohydrates, 4.9 g of protein and 2.3 g of fat. Percentage of total energy from carbohydrate was 60%, from protein 18% and from fat 19%. Participants were given a large bowl with a single weighed portion of approximately 1300 g and a 330 mL bottle of still water. The participants were seated again in the same quiet room as for the rice meals and they were told that the portion provided was deliberately much larger than that normally consumed, and to eat from the bowl until satisfied, as used previously (157, 189). They were instructed to drink the water with the pasta meal as they wanted. The amount of pasta left over was removed

and weighed. The type of meal was selected as it would allow each spoonful to provide homogenous composition of ingredients, having been carefully stirred. The energy intake was calculated from the amount consumed as an objective measure of food consumption. The speed of the *ad libitum* meal consumption was measured by dividing the amount of pasta intake in g on the time taken to eat it in minutes (158)

Figure 4.5 Image of the packaging of the tomato and mozzarella pasta bake (Tesco supermarket, UK) used for the *ad libitum* pasta meal.

4.2.3.4 Total daily energy intake

A food diary was given to the participants at the end of each study day. They were asked to keep a detailed record of food and beverages consumed over the reminder of the day to enable calculation of the daily energy intake as described in section 2.3.5.

4.2.3.5 Gastric volume and small bowel water measured by MRI

Gastric volumes, GE and SBWC measurements were carried out as described in the methods Chapter, Section 2.4.

4.2.4 Data and statistical analysis

Sample size calculation

We did not have data on glucose responses to similar LAGG with rice meals to carry out a formal power calculation. We based instead our N=12 sample size selection on the paper by Fuwa *et al.* (137) which used a similar study design. They showed a statistically significant reduction in postprandial blood glucose responses studying N=11 healthy humans who were fed a different white rice variety (Japonica Koshiibuki rice) cooked with the addition of a different gum (xanthan) compared with the same rice without the added gum.

Data presentation and analysis

Descriptive data were presented as means with the standard error of the mean (SEM) unless stated otherwise.

The blood glucose data was collected on hard copy CRF then transcribed to a Microsoft Excel sheet. The 2-hours iAUC was calculated for blood glucose for each participant and arm of the study. The iAUC is commonly used for glycaemic response data calculation (190) and it ignores the

area of the curve beneath the fasting concentration (191). For all the other data, the total AUC was calculated, when appropriate, from the respective time curves for each participant and arm of the study without subtracting baseline values. The Shapiro-Wilk normality test was used to test for normal distributions. This confirmed a paired t-test was appropriate to compare AUCs for most data sets, apart from the stomach gas volume data for which a Wilcoxon match-paired signed rank test was used.

Two-way repeated measures ANOVA were undertaken to compare time courses. When an interaction was identified, simple effects of treatment were explored by comparing the feeds at each timepoint applying a Bonferroni correction for multiple testing.

Data was analysed using GraphPad Prism for windows version 9.2.0 (GraphPad Software, San Diego, California). Statistical significance of differences was assumed with a P value less than 0.05.

4.3 Results

Twelve participants completed the human study, seven female and five male. They had a mean age of 26±2 years; BMI of 23±1 kg/m^2; weight of 72 ± 3 kg and height of 175 ± 3 cm, Table 4.1 shows the demographic data by sex. All participants consumed the allocated two rice meals within the time required. The study procedures were tolerated well and there were no adverse events reported during the study.

Table 4.1 Demographic data by sex for the study participants.

	Male n=5	Female n=7
Age (years)	21± 0	29 ± 3
Weight (kg)	83 ± 2	64 ± 2
Height (cm)	183 ± 3	169 ± 2
BMI (kg/m^2)	25 ± 1	22 ± 1

4.3.1 Blood glucose

Twelve complete data sets were available for analysis for all the study outcomes save for the food diaries, which had one fewer data set since one participant failed to return a questionnaire. The time courses for the group means for finger prick blood glucose are shown in Figure 4.6. The baseline values were within the healthy normal range and there was no baseline difference between arms of the study, being 5.1 ± 0.1 mmol/L for the rice control meal and 5.1 ± 0.1 mmol/L for the rice + LAGG meal ($P > 0.9999$).

After this time the blood glucose values gradually decreased but at the end of the sampling period at T=120 minutes had not returned to baseline values. Numerically, the values for the rice + LAGG meal remained consistently lower than those for the rice control meal. The largest mean difference in glucose values from control was observed at T= 75 with a value of 0.8 ± 0.2 mmol/L.

The iAUC 2h for finger prick blood glucose was the principal outcome of this study. The iAUC for blood glucose for the white rice + LAGG was 93 ± 16 mmol/L · min whilst the iAUC for the plain white rice was 160 ± 18 mmol/L· min. This corresponds to a percentage difference of 9.1%. The difference in iAUC was highly significant (Table 4.2).

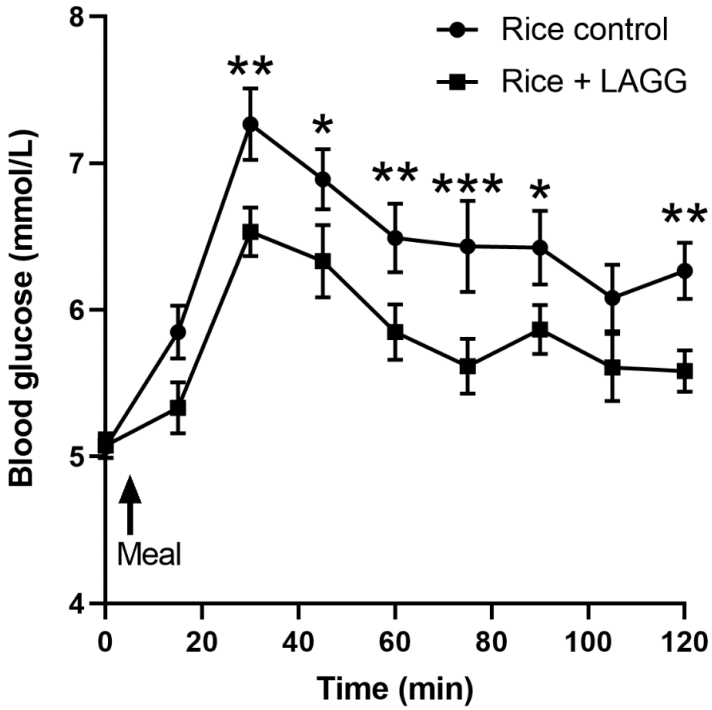


Figure 4.6 Blood glucose time courses from T=0 to T=120 minutes for N=12 participants who consumed the test meal with and without the addition of low acyl gellan gum (LAGG). Data points are mean ±standard error of the mean. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

The individual blood glucose levels rarely went below baseline and therefore the significant differences revealed by the iAUC 2h calculation

(baseline corrected and ignoring negative areas below baseline) are unchanged when using a simple AUC 2h trapezoidal integral (total AUC with no baseline correction) as shown in Table 4.2. Two-way repeated measure ANOVA analysis showed no interaction of rice meal type × time ($P = 0.1210$).

Exploring the main effects, there was a significant effect of rice meal type ($P < 0.0001$) and a significant effect of time ($P < 0.0001$). Following this, exploratory *post-hoc* analysis of blood glucose means for each time point corrected for multiple comparisons showed significant postprandial differences at all time points apart from T=15 min and T=105 min (Figure 4.6).

Blood glucose rose postprandially to a peak at T=30 minutes, with the rice peak 7.3 ± 0.2 mmol/L significantly higher than for the rice + LAGG meal 6.5 ± 0.2 mmol/L ($P < 0.01$).

4.3.1 Subjective appetite responses

Figures 4.7 to 4.12 show the time courses for individual and composite subjective appetite responses to the two rice test meals. The scores for hunger (Figure 4.7), desire to eat (Figure 4.10) and PFC (Figure 4.11) decreased when the participants consumed the rice meals. with the LAGG.

Table 4.2 Summary results table for N=12 participants who consumed the test meal with and without the addition of low acyl gellan gum (LAGG). Data are shown as mean ±standard error of the mean.

	Rice control	**Rice + LAGG**	***P value***
Glucose iAUC 2h (mmol/L·min)	160 ± 18	93 ± 16	0.0007
Glucose AUC 2h (mmol/L·min)	767 ± 20	697 ± 17	<0.0001
Gastric meal volume AUC 2h (mL·min)	32340 ± 1831	30914 ± 1077	0.3959
Gastric meal volume half emptying time T50% (min)	107 ± 12	101 ± 6	>0.99
Gastric meal volume emptying rate (mL /min)	2.1 ± 0.2	2.0 ± 0.1	0.8598
Gastric gas volume AUC 2h (mL·min)	2772 ± 507	1851 ± 237	0.1099
Total gastric meal volume AUC 2h (mL·min)	35113 ± 1862	32765 ± 1196	0.1892
Gastric total volume half emptying time T50% (min)	109 ± 11	106 ± 7	0.8240
Gastric total volume emptying rate (mL /min)	2.1 ± 0.2	2.1 ± 0.2	0.6009
Small bowel water content AUC 2h (mL·min)	2939 ± 454	2020 ± 260	0.0253

Composite appetite score AUC 2h (mm·min)	6076 ± 584	5729 ± 493	0.2666
Hunger AUC 2h (mm·min)	5186 ± 658	5080 ± 565	0.7398
Satisfaction AUC 2h (mm·min)	5950 ± 541	6139 ± 578	0.5762
Fullness AUC 2h (mm·min)	5683 ± 564	6140 ± 548	0.2945
Desire to eat AUC 2h (mm·min)	5901 ± 806	5552 ± 627	0.4587
Prospective consumption AUC 2h (mm·min)	6923 ± 565	6289 ± 652	0.1738
Amount of *ad libitum* lunch meal eaten (g)	577 ± 65	579 ± 74	0.9680
Energy intake from *ad libitum* lunch meal (kcal)	629 ± 70	631 ± 81	0.9680
Time taken to eat *ad libitum* lunch meal (min)	10 ± 1	10 ± 1	0.9292
Speed of eating *ad libitum* lunch meal (g/min)	57 ± 6	59 ± 8	0.6480
Self-reported energy intake for the reminder of the day (kcal) (*n*=11)	1435 ± 166	1176 ± 210	0.1601
Self-reported carbohydrate's intake for the reminder of the day (g) (*n*=11)	151 ± 20	139 ± 22	0.7249

Self-reported fat intake for the reminder of the day (g) (*n*=11)	55 ± 10	45 ± 10	0.1445
Self-reported protein intake for the reminder of the day (g) (*n*=11)	80 ± 14	55 ± 12	0.0068
Total energy intake for the whole day (kcal) (*n*=11)	2329 ± 258	2064 ± 206	0.1606

The self-reported intakes are reported for n=11 as 1 food record questionnaire was not returned. Abbreviations: AUC 2h: area under the curve for 2 h.

Visually, the decrease from baseline was greater with rice plus LAGG meal than without the LAGG. However, the differences between the areas under the curves of the appetite rating score for hunger, desire to eat and PFC were not statistically significant (Table 4.2).

Two-way repeated measure ANOVA analysis for hunger VAS scores showed no significant interaction of rice meal type × time (P = 0.7830), no significant main effect of rice meal type (P = 0.8242) and a significant main effect of time (P < 0.0001) with Two-way repeated measure ANOVA analysis for desire to eat VAS scores showed no significant interaction of rice meal type × time (P = 0.3237), no significant main effect of rice meal type (P = 0.5236 and a significant main effect of time (P < 0.0001). Two-way repeated measure ANOVA analysis for prospective

consumption VAS scores showed no significant interaction of rice meal type × time (P = 0.4604), no significant effect of rice meal type (P = 0.1894) and a significant effect of time (P < 0.0001).

The score for the fullness (Figure 4.9) and satisfaction (Figure 4.8) increased when the participants consumed the rice meals. Numerically the increase was greater with the rice plus LAGG meal compared to rice control meal. However, the difference between areas under the curves of fullness and satisfaction were not statistically significant (Table 4.2). Two-way repeated measure ANOVA analysis for satisfaction VAS scores showed no significant interaction of rice meal type × time (P = 0.6442), no significant effect of rice meal type (P = 0.5173) and a significant effect of time (P < 0.0001). Two-way repeated measure ANOVA analysis for fullness VAS scores showed no interaction of rice meal type × time (P = 0.7184), no significant effect of rice meal type (P = 0.2827) and a significant effect of time (P < 0.0001).

The time courses for the composite appetite score are shown in Figure 4.12. The difference of the AUC overall composite score was not statically significant for the two meals being 6076±584 for rice and 5729±493 for rice +LAGG (P = 0.2666). Two-way repeated measure ANOVA analysis showed no interaction of rice meal type × time (P = 0.7123), no significant effect of rice meal type (P = 0.2804) and a significant effect of time (P < 0.0001).

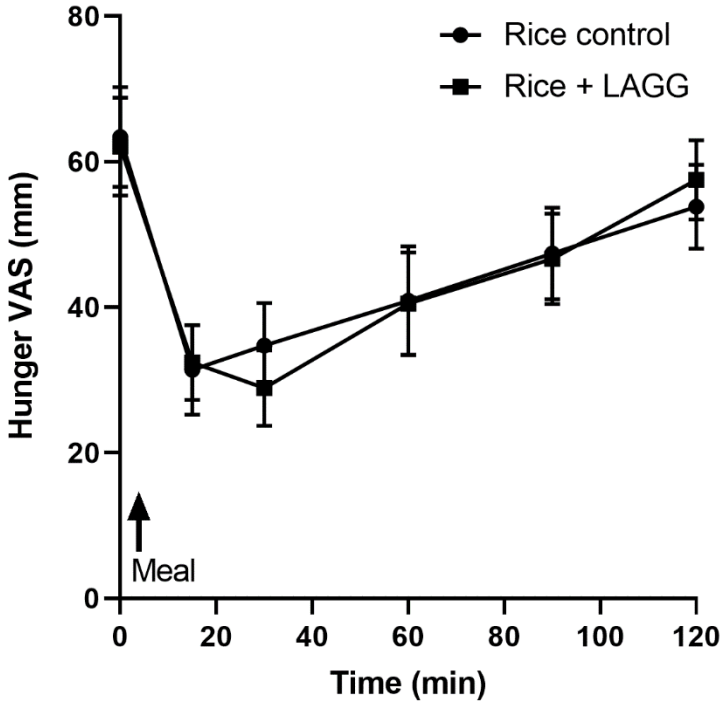


Figure 4.7 Hunger visual analogue scale (VAS) time courses from N=12 healthy participants who consumed the rice meal with and without the addition of low acyl gellan gum (LAGG) during the cooking process. Data points are mean ± SEM.

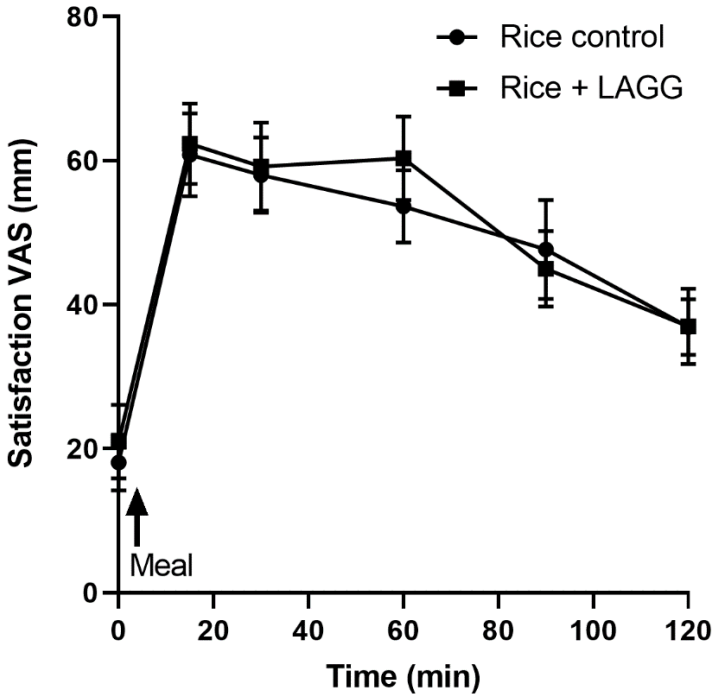


Figure 4.8 Satisfaction visual analogue scale (VAS) time courses from N=12 healthy participants who consumed the rice meal with and without the addition of low acyl gellan gum (LAGG) during the cooking process. Data points are mean ± SEM.

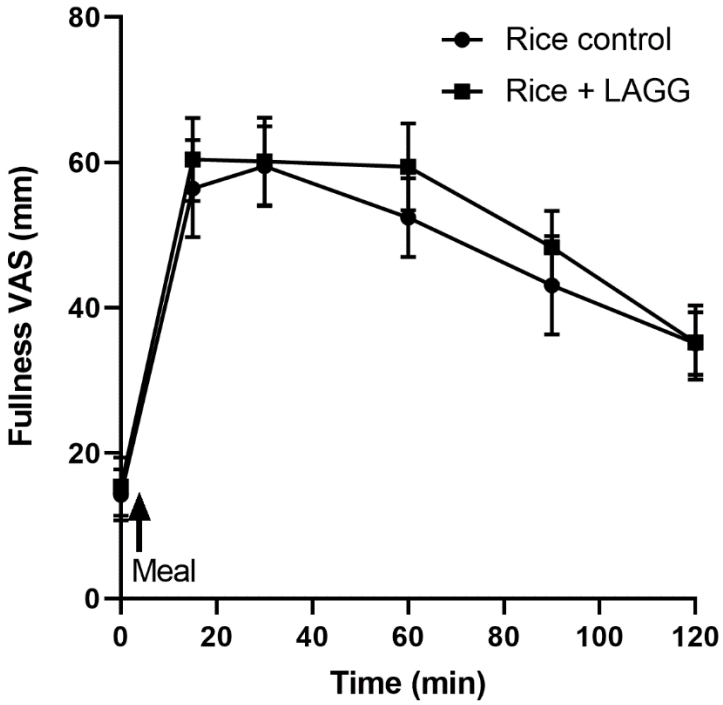


Figure 4.9 Fullness visual analogue scale (VAS) time courses from N=12 healthy participants who consumed the rice meal with and without the addition of low acyl gellan gum (LAGG) during the cooking process. Data points are mean ± SEM.

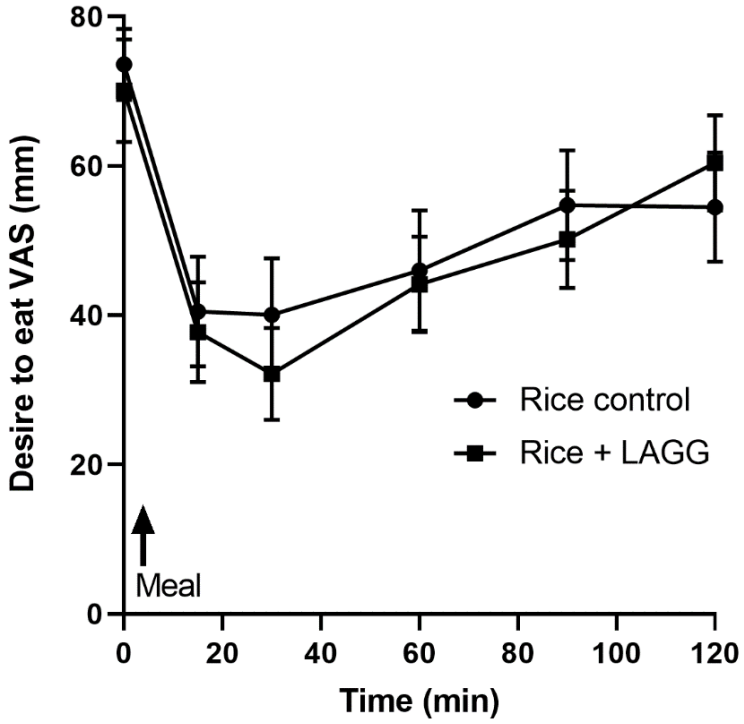


Figure 4.10 Desire to eat visual analogue scale (VAS) time courses from N=12 healthy participants who consumed the rice meal with and without the addition of low acyl gellan gum (LAGG) during the cooking process. Data points are mean ± SEM.

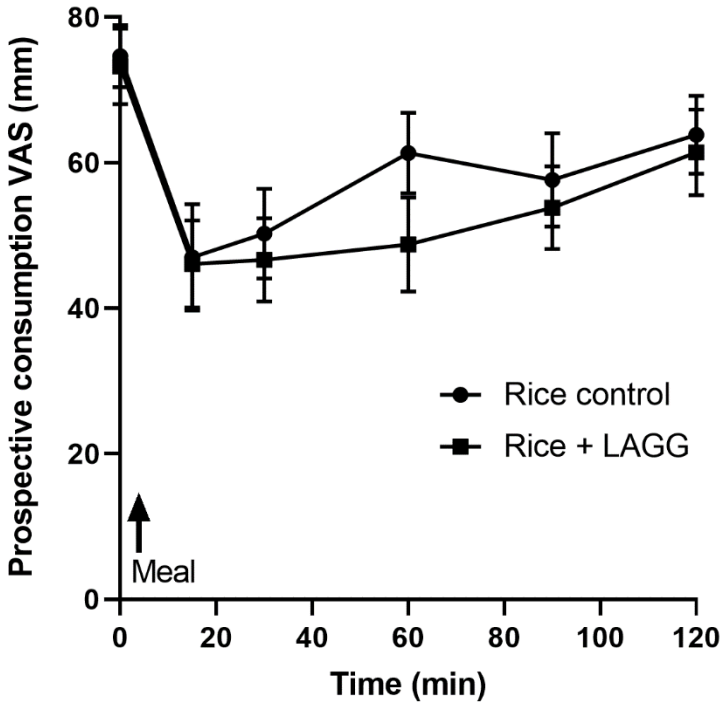


Figure 4.11 Prospective consumption visual analogue scale (VAS) time courses from N=12 healthy participants who consumed the rice meal with and without the addition of low acyl gellan gum (LAGG) during the cooking process. Data points are mean ± SEM.

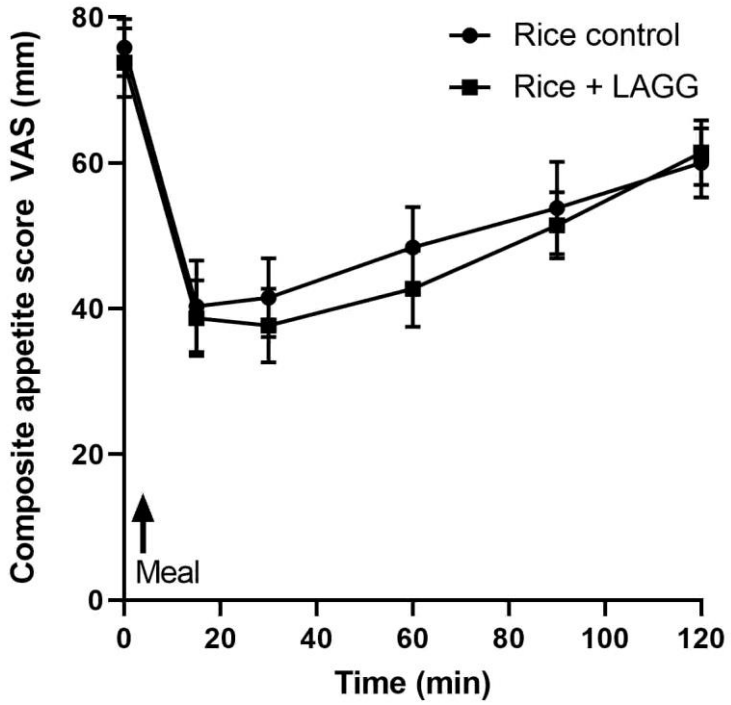


Figure 4.12 Composite appetite score visual analogue scale (VAS) time courses from N=12 healthy participants who consumed the rice meal with and without the addition of low acyl gellan gum (LAGG) during the cooking process. Data points are mean ± SEM. Lower values in this context indicate lower hunger, higher fullness and less desire to eat, whilst higher values indicated the opposite (53, 88)

4.3.2 Satiety quotient

The SQ time courses for the composite appetite score are shown in Figure 4.13. The SQ time courses for the feelings of hunger, satisfaction, fullness, desire to eat and prospective consumption are shown in Appendix 8.8.

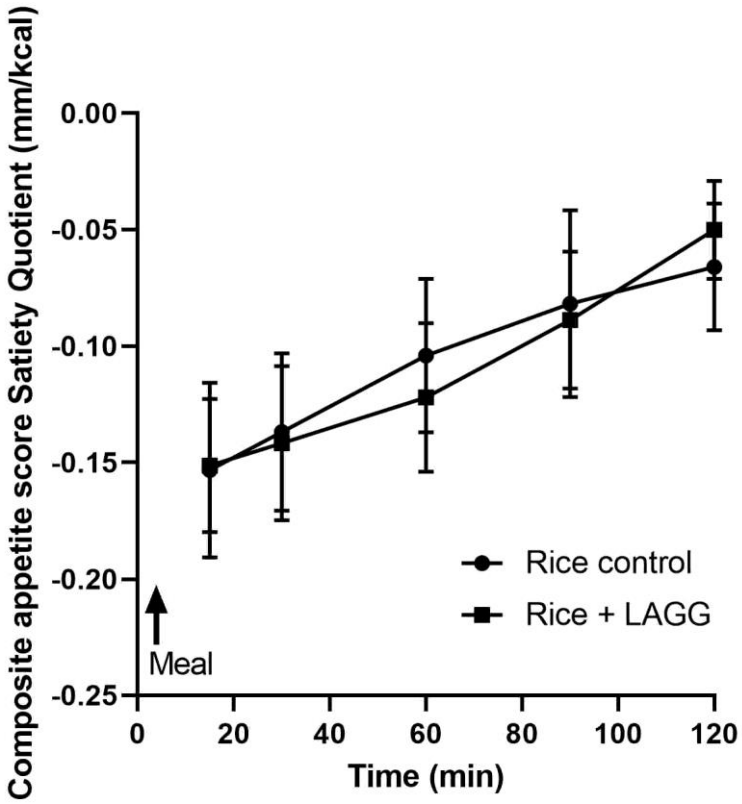


Figure 4.13 Composite appetite score Satiety Quotient time courses from N=12 healthy participants who consumed the rice meal with and without the addition of low acyl gellan gum (LAGG) during the cooking process. Data points are mean ± SEM.

4.3.3 *Ad libitum* meal

The amount consumed from the *ad libitum* meal was 577±65 g for the rice control and 579±74g for the rice + LAGG meal and the two amounts were not significantly different (P = 0.9680).

The time taken to consume the *ad libitum* meal for both the groups was 10±1 min. The mean rate of eating of the *ad libitum* lunch meal (g/min) was 57 ± 6 g/ min for the rice control group and 59 ± 8g/ min for the rice+

LAGG group which was not a statistically significant difference (*P* = 0.6480).

The final composite appetite score after the *ad libitum* meal was 9±2 for rice control and 10±1 for the LAGG rice group and the difference between groups was not significant (*P* = 0.8254).

4.3.4 Food Records

One food record was not returned. The mean energy intake from the self-reported dietary intake records following the consumption of the rice meal was 2329 ± 258 kcal/day and the rice with LAGG meal was 2064 ± 206 kcal/day. However, this difference was not statistically significant. (*P* = 0.1606).

4.3.5 Intragastric rice meals appearance and volumes

Firstly, all the MRI scans were reviewed qualitatively to explore what the appearance of the rice meals was in the stomach and if any characteristics could be noted. This task was undertaken with the operator blind to the meal type.

After ingestion, the rice meals inside the stomach provided good signal on the moderately T2 weighted scans (Figure 4.14). The rice meal had a characteristic grainy appearance. Intragastric fluids (e.g. secretion, water from the drink) appeared brighter than the rice and surrounding organs and formed a fluid layer on top of the rice. A small amount of black intragastric gas was often present on top of the water layer. During the

course of digestion, the rice control appeared to become brighter and to lose the grainy appearance, becoming more homogeneous chyme with time than immediately after ingestion. Some water layer on top of the meal remained present for part of the time course but was mostly gone by the final scan at 120 minutes. Within the rice only meal some boluses could be identified after ingestion in 9 of the participants but these boluses were only visible at later time points in 2 of the 12 participants (Table 4.3).

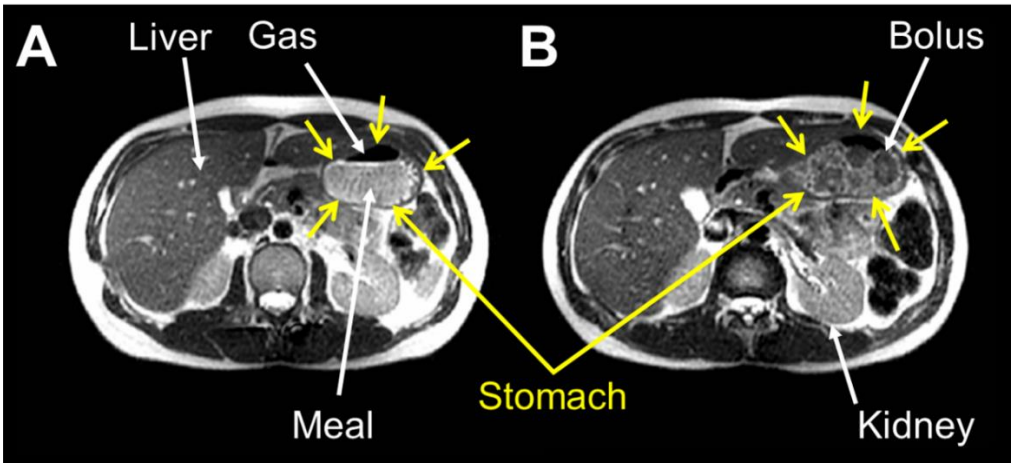


Figure 4.14 (A) MRI image acquired axially through the stomach of a study participant at T=90 minutes after feeding the rice control meal. (B) Corresponding MRI image from the same participant taken at the same time point but after consuming the rice + low acyl gellan gum (LAGG) meal. In (B) darker, round boluses are visible inside the stomach. Anatomical landmarks such as the liver and kidneys are also indicated by white arrows for ease of orientation.

Conversely the rice + LAGG remained consistently darker, and some grainy appearance persisted throughout. Clearly defined, darker, multiple rice boluses (Figure 4.14 B) could be seen in all 12 participants after ingestion and were still visible at later time points in 11 of them.

Table 4.3 Summary table indicating whether rice boluses were identified (green tick mark) or not (red cross mark) inside the stomach of each of the 12 healthy volunteers immediately after feeding and at later time points up to 2 hours after feeding.

	Rice control		**Rice + Gellan**	
Participant	Boluses visible after feeding	Boluses visible at later time points	Boluses visible after feeding	Boluses visible at later time points
1	✓	✗	✓	✗
2	✓	✗	✓	✓
3	✓	✓	✓	✓
4	✗	✗	✓	✓
5	✓	✗	✓	✓
6	✗	✗	✓	✓
7	✓	✗	✓	✓
8	✗	✗	✓	✓
9	✓	✗	✓	✓
10	✓	✓	✓	✓
11	✓	✗	✓	✓
12	✓	✗	✓	✓

4.3.5.1 Gastric meal volume

The meal (chyme, including water and rice meal) and gas contents of the stomach were outlined in the MRI images for volume measurement as they provided good contrast against surrounding organs.

The MRI baseline images allowed a check that all participants had come in fasted for their study day, which was confirmed. The stomach at baseline contained only a small volume of resting gastric juices, averaging 52 ± 13 mL for the rice control meal, and 40 ± 6 mL for the rice + LAGG meal, with no significant difference (P = 0.9259).

The average time course for gastric meal volume (excluding gas, as presented in the remainder of this section) is shown in Figure 4.15.

Gastric meal volumes rose postprandially at T=15 min to approximately the same average volume, 412 ±17 mL for rice and 420 ± 16 mL for rice with LAGG with no significant difference (P = 0.9852). Subsequently, gastric meal volume declined, initially with a higher rate for the rice + LAGG meal up to T=30. The gastric meal volume difference with time between the two meals decreased until T=120 when both average meal volumes converge to around 182 mL. By T=120 min the gastric meal volume had not yet returned to the fasting baseline volumes.

The 2-hours AUC for gastric meal volume for the rice + LAGG meal was 30914 ± 1077 mL·min, modestly and not significantly smaller than that of the rice control meal 32340 ± 1831 mL·min (P = 0.3959).

For the gastric meal volumes, the 2-way repeated measure ANOVA analysis showed no interaction of rice meal type × time (P = 0.4287),

no significant effect of rice meal type (P = 0.4337) but a significant effect of time (P < 0.0001).

The shape of the individual GE curves was borderline between linear and exponential as shown by the respective goodness of fits R^2 parameter being quite close between the two fits. The gastric volume emptying curves were primarily exponential (83% of the emptying curves fitted best for exponential curve) and the fits were overall good, with average R^2 = 0.92 ± 0.02.

The AUC 2h for the gastric meal volume for white rice with and without LAGG are shown in Table 4.2. There was a modest difference between these two values and that was not significant. Extrapolating the curves, one can estimate that GE would be complete in around 180 – 210 minutes in total.

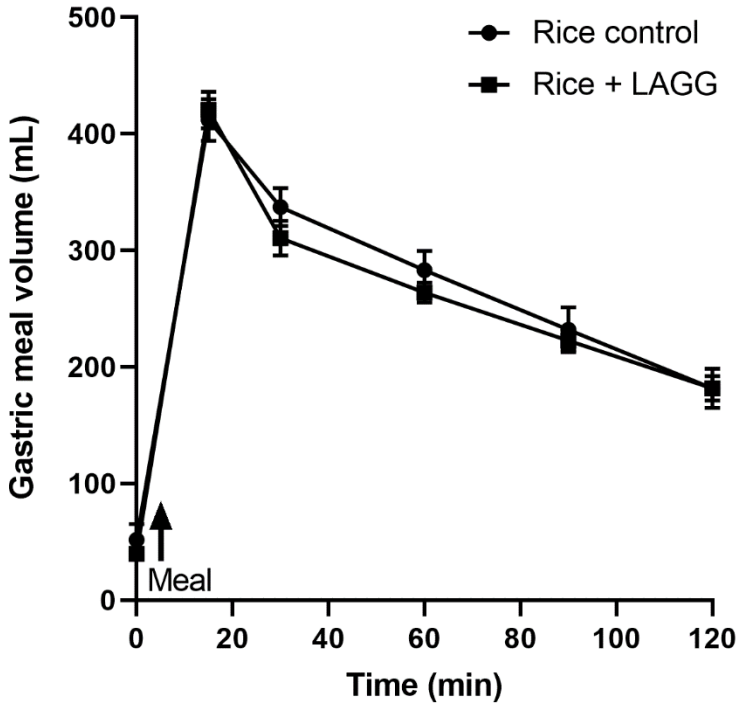


Figure 4.15 Gastric meal volume time courses from N=12 healthy participants who consumed the rice meal with and without the addition of low acyl gellan gum (LAGG) during the cooking process. Data points are mean ± SEM.

The half GE time T50% for the meal (excluding gas volume) was not significantly different between rice control 107 ± 12 min and rice plus LAGG 101 ± 6 min (paired Wilcoxon's $P > 0.99$). The meal volume emptying rate was also not different between rice control 2.1 ± 0.2 mL/min and rice plus LAGG 2.0 ± 0.1 mL/min (paired t test $P = 0.8598$).

4.3.5.2 Gastric gas volumes and total gastric volumes

The time courses for gastric gas volume and total gastric volume are shown respectively in Figure 4.16 and Figure 4.17. Gastric gas volumes were also measured individually from the MRI images. It was not possible to calculate an emptying time or rate for the gastric gas volume. Looking at the individual volume/time curves, the gas volume did not exhibit a recognisable behaviour with time but it varied without a recognisable pattern, within an average band of between 15 mL and 30 mL of intragastric gas. For most participants the gas volume did not change much from baseline, and for a smaller number of participants it moderately increased and for some moderately decreased after feeding.

The AUC for stomach gas for the rice control meal 2772 ± 507 mL·min and 1851 ± 237 mL·min for the rice + LAGG meal, with no significant difference (P = 0.1099). Given the small amount of intragastric gas present, when the individual gas volumes were added to the individual meal volumes at each time point to calculate total gastric volume (meal plus gas), the increase in volume was only modest and the results for GE time and rate did not change significantly. Total gastric volume T50% was 109 ± 11 min for the rice control meal not different from the total gastric volume T50% for the rice plus LAGG meal which was 106 ± 7 min (paired t test P = 0.8240). Total gastric volume emptying rate was 2.1 ± 0.2 mL/min for the rice control meal again not different from that for the rice plus LAGG meal 2.1 ± 0.2 mL/min (paired t test P = 0.6009).

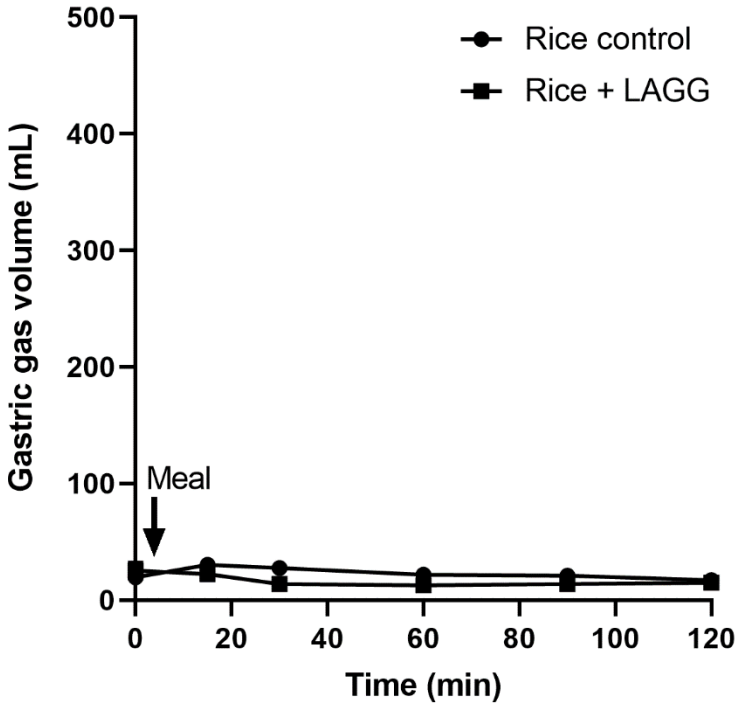


Figure 4.16 Gastric gas volume time courses from N=12 healthy participants who consumed the rice meal with and without the addition of low acyl gellan gum (LAGG) during the cooking process. Data points are mean ± SEM.

Adding the gastric meal and the gastric gas volumes yielded the total gastric volume (chyme + gas) which, due to the small amount of gas present, is only marginally different from the gastric meal volumes. Gas volumes were consistently low averaging between 14 mL and 31 mL across the time points for both rice meals. As such, adding the gas volumes to the rice meal volumes to obtain the total gastric volume did not make notable changes to the curves shown in Figure 4.17.

The total gastric volume AUC for the rice + LAGG meal was 32765 ± 1196 mL·min compared to that for the rice control meal 35113 ± 1862 mL·min with no significant difference *(P* = 0.1892).

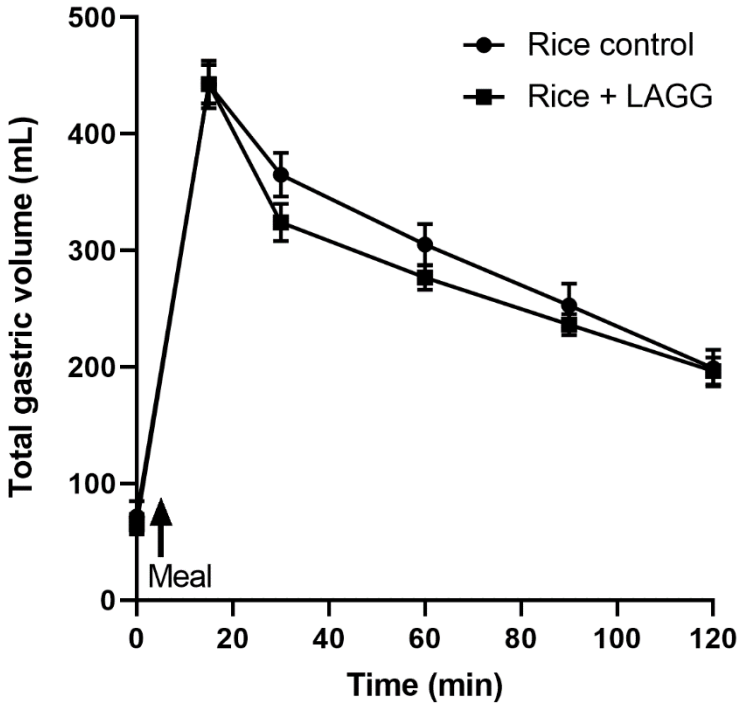


Figure 4.17 Total gastric volume (meal volume plus gas volume) time courses from N=12 healthy participants who consumed the rice meal with and without the addition of low acyl gellan gum (LAGG) during the cooking process. Data points are mean ± SEM.

4.3.6 Small bowel water content

The time courses for SBWC are shown in Figure 4.18. Small bowel water decreased after feeding and there was a modest but significant difference between the two meals with the rice control values being higher than those for rice + LAGG. There was no significant interaction

term in the 2-way ANOVA. Main effect of meal type was significant (P = 0.0337) and also effect of time was significant (P < 0.0001). *Post-hoc* analysis showed that the values at T=15 were significantly different after correction for multiple comparison (P < 0.05).

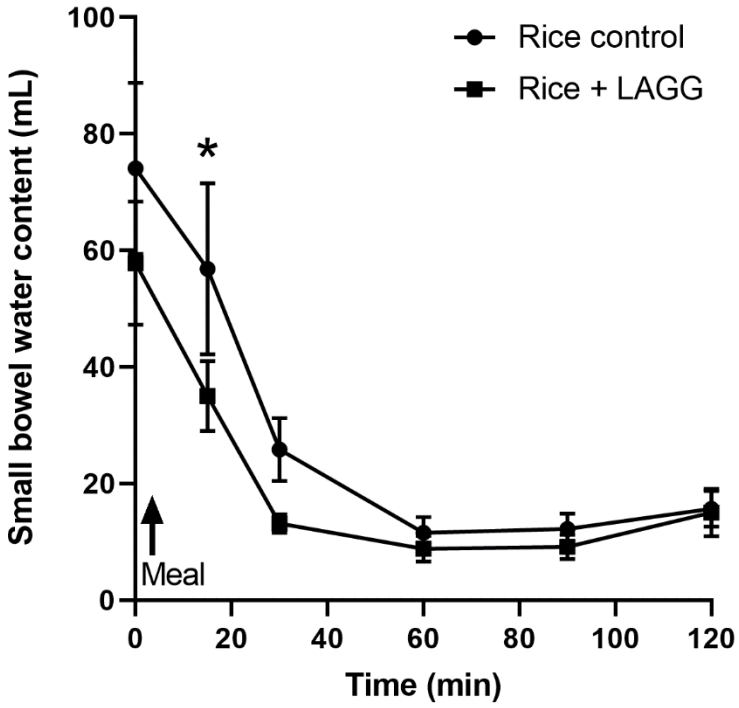


Figure 4.18 Small bowel water content time courses from N=12 healthy participants who consumed the rice meal with and without the addition of low acyl gellan gum (LAGG) during the cooking process. Data points are mean ± SEM. Significant *post-hoc* 2-way repeated measures ANOVA, Bonferroni corrected, pairwise comparisons at each time point versus rice control meal (*P < 0.05).

4.3.1 End of study questions

At the end of their last visit, all 12 participants answered ‘yes’ to the question if the study was acceptable and ‘yes’ to the question if the meals were acceptable. Four out of 12 participants answered ‘yes’ to the question if they could perceive a difference between the two rice meals.

4.4 Discussion

The main aim of this study was to test the effect of LAGG addition, when cooking high GI jasmine white rice, on blood glucose response, appetite response and gastrointestinal response. The study was successful in collecting the blood glucose data, the completed VAS questionnaires and the MRI images of the stomach for white rice and rice with the addition of LAGG.

The experimental procedures were well received by the participants and no dropouts occurred.

Data from this study confirmed that the main hypothesis was correct. Adding LAGG to cooking water significantly reduced the post prandial blood glucose levels in our healthy participant population. These findings are compatible with those of a previous study that found that xanthan gum effectively reduced blood glucose levels after consumption of a different variety of rice (137).

This composite appetite score is increasingly used in the literature, as it integrates different dimensions of appetite sensations into one single

index. Higher composite appetite scores are associated with higher appetite feeling. Researchers use VAS scales to quantify appetite feelings. Subjective ratings using VAS questionnaires, particularly within subjects, have generally been shown to be reproducible, reliable and sensitive indicators of appetite (88). In this study the mean composite appetite score over the postprandial period for rice + LAGG was lower than for rice control meal indicating lower feelings of appetite though this difference was not significant. These findings are compatible with the non–significant lower energy intake for the reminder of the day (approximately 300 kcal less for the rice + LAGG meal). The lack of statistical significance may be due to lack of power for these secondary outcomes. Paradoxically, stomach volumes, a secondary end point, did not show a significant difference. Indeed, meal volumes for the rice + LAGG arm of the study were numerically lower than those for the rice control between 30 min and 90 min postprandially, hence would result in less satiety due to less distension of mechanoreceptors in the gastric wall However it is recognised that many other factors impact on appetite.

Duodenal energy feedback may also have played a role as the rice + LAGG is likely to have delivered energy to the duodenum slower than the rice control. It has been shown that meal energy content can modulate GE with lower energy content emptying faster (192) whilst higher energy content can delay GE (193).

Gastric meal volume T50% and gastric meal volume emptying rate were not significantly different between meals indicating that the delivery of chyme to the duodenum from both meals was similar, and that therefore

overall gastric emptying rate was not part of the mechanism underlying the differences in blood glucose levels. Only a small amount of intragastric gas was present, and this would not have affected stomach volumes not stimulated differently the stomach's stretch receptors.

SBWC is a key parameter that allows insights into small intestinal function and its response to foods. SBWC depends on small bowel motility, gastric emptying of nutrients from the stomach, and the balance of secretion and absorption of fluids in response to the feeding intervention (194). Natural fibres and particulate have been shown to stimulate SBWC (195) as well as plastic particles (196) and therefore there was interest in exploring the effect of rice on the content of water of the small bowel in this acute intervention study. There was an initial fall from baseline values which was indicative of the stimulatory effect of available glucose on water absorption, including also an effect of the ileum emptying in response to the meal (194). The differences observed in SBWC curves between rice control and rice plus LAGG were modest. Our experimental duration of two hours allowed measurement of the 'gastric phase' up to about 90 minutes into the postprandial period. Based on the literature (197) the 'intestinal' phase with increased fluid volumes occurs after this period and therefore a rise in bowel water volumes could not be observed here. A possible mechanisms for this apparently quicker reduction in gastric meal volume with LAGG GE could be gastric sieving. Gastric sieving is the preferential emptying of fluids from the stomach when it contains both solids and liquid separately (36). Visual, qualitative inspection of the MRI images seemed to show that the

water layer resting on top of the rice particulate disappeared quicker for the LAGG arm of the study and this is worth investigating further in future studies. However, in conjunction with the similar gastric meal volumes and emptying rates it would appear that that bolus formation and retention did not significantly drive sieving, with part of the stomach fluid being utilized to hydrate the meals further.

4.4.1 Strengths of the study

The 3% LAGG dose was chosen based on the *in vitro* digestion studies presented in the Chapter 2, showing the importance of characterising *in vitro* and using the outcomes to select the interventions for the *in vivo*, and much more expensive, studies.

The baseline finger prick sample taken at the study day confirmed the screening values and that all participants were healthy and had fasted before coming in for the study day. In addition, great care was taken to minimise confounding by carefully controlling the conditions of the preparation, presentation and consumption of the two intervention meals. The participants were asked to eat the same meal (of their choice) for dinner before each of the two study days to reduce variability and compliance with this was checked verbally at the study day. Although the rest of the diet on the day before the glucose study was not controlled participants were asked to avoid strenuous exercise and alcohol for 24 hours prior to the study as this can affect blood glucose levels (198).

4.4.2 Limitations

This is the thought to be the first study to consider then impact of LAGG hence no directly relevant data was available when calculating sample size. The sample size thus was based on a similar study by Fuwa et al (137) for the main outcome glucose iAUC. The 12 participants studied were sufficient to detect a significant difference for the blood glucose iAUC. However, the study was not powered to detect differences in the other outcomes. Particularly, the satiety outcomes appeared to be showing a tendency for potentially 'beneficial' results if aiming to suppress appetite. In future studies it would be beneficial to consider a larger sample size to test more fully the appetitive effects of rice + LAGG.

The participants were all healthy and mostly young, so it would be beneficial to know if the LAGG had similar or even greater effects on participants if they were people living with diabetes or from an older cohort. The participants were also predominantly Caucasian, whilst it has been shown that Asian people may respond differently to rice digestion (199). Glycaemic responses have also been shown to be higher in Chinese participants compared with Europeans after exposure to rice or glucose (199).

Use of the fingerpick blood tests measured capillary blood glucose levels. It is known that there is a small difference between blood glucose levels taken from capillary compared with venous sources (154), Venous blood is more likely to be influenced by local uptake by tissue/muscle hence it may give a less accurate reflection of whole body glucose metabolism.

The study set up and funding did not allow for collecting blood and measuring hormone peptides. Without the data from key hormones regulating glucose metabolism such as for example insulin, glucagon, GIP and GLP-1, it is difficult to speculate on physiological mechanisms for the observed glycaemic responses. The finger prick blood test was however a sensible alternative to use, it was relatively cheap, and caused only a short, sharp scratch with only a transient small discomfort for the participants as opposed to the vein cannulation procedures with the use of a hot box to enable collection of arterialised blood.

The postprandial duration of this study was 2 hours. This was chosen as the standard time frame for measuring the GI of foods (156). Looking at the time curves from this study the blood glucose and stomach volumes had not yet gone back to fasting baseline values at the 2 hour time point. The curves can be extrapolated, showing that a longer time window to 180-210 min would be more appropriate to capture the return to baseline of the study endpoints. This can inform the following study.

For the *ad libitum* meal more pasta was provided to the participants than would usually be consumed and this was conveyed to the participants. This was to avoid the unconscious aim to finish off a smaller portion. Other methods could be used in future studies, for example an alternative technique would have been to have provided a series of portions, replacing a portion when approximately 3/4 had been consumed so that the amount eaten did not act as a cue (the ‘Bottomless bowl’ technique) (200).

In assessing the total energy intake for the day, use of a food diary, may have introduced under or over reporting (201). This was minimised by training the participants in the use of the diary and would be less likely to occur in this participant group, than in those who are living with obesity (201).

Looking at the MRI part of the study, a limitation was that the participants were imaged in the supine position, which is not the more physiological upright position for digestion. However, the participants were only lying in the machine for approximately 8 minutes at each scanning time, so this is unlikely to have had a strong effect, and the positioning of the participants was the same for both arms of the study.

Another limitation was that using MRI made the study more expensive and limited the number of participants that could be studied on each day. It was originally planned to interleave two participants per morning to make the use of the MRI scanner time more effective. However, due to the COVID 19 pandemic and the change in the risk assessments at the time this study was finally run, only one participant could attend on each study day. This doubled the length of time and the cost to run this study.

Another limitation was that the MRI data was analysed using manual drawings of ROIs which was a laborious and lengthy process. If the study was to be repeated using MRI imaging it may be worth adjusting the MRI parameters to obtain a stronger T2 weighting and a better contrast between meal and surrounding organs, which may allow computer software to draw the ROIs more automatically.

4.5 Conclusions

This study was successful. It showed that the addition of LAGG to the cooking process of white jasmine rice resulted in a significant decrease in postprandial blood glucose levels compared with plain white rice, after an acute dose in a healthy adult population. This is an exciting finding but whether the effect would be sustained after a repeated (chronic) exposure remains to be determined.

5 The effects of LAGG on the acute glycaemic and appetitive responses to a white rice meal and impact on energy intake over a 7-day period.

Building on the previous study, the work described in this chapter aims to evaluate if the effects of adding LAGG to jasmine rice processing on acute postprandial blood glucose response are sustained, after a repeated daily intervention for 7 days.

5.1 Introduction

Data shown in Chapter 3 indicated that the addition of 3% (w/w of dried rice) LAGG to jasmine white rice during the cooking process had a significant effect on lowering blood glucose response. The LAGG group also showed a tendency for lower appetite and lower energy intake.

However, that study examined only one acute exposure to the rice + LAGG intervention. Single intervention feeding studies are very common to investigate specific effects or mode of action of foods, beverages or ingredients. They cannot demonstrate however whether any 'beneficial' effects shown on physiological and/or behavioral responses would persist or decline after a repeated exposure to the dietary intervention over several days. The human body can adapt to physiological processes and learning can occur. Such adaptation may mean that the responses observed in an acute setting may diminish or be amplified following repeated exposures to the food over time. Changing to a high

fat diet, for example, has been shown in mice to result in enterocytes upregulating lipid absorption genes and showing increased lipid absorption *in vivo* over 7 days, with changes apparent after one day (202). The microbiota too is influenced by shifts in response to acute dietary interventions but whether these responses are durable remains to be investigated (203). It is therefore important to assess the sustained effect of the diet intervention over a number of days in order to be establish the stability of the acute effect seen (204). The number of days considered to be adequate when testing a repeated exposure is unclear. The European Food Safety Authority (EFSA) states that evidence for a sustained effect with continuous consumption of the food should be demonstrated in order to prove health claims related to that food but does not specify the duration for studies of appetite or energy intake claims (205). The UK SACN also does not recommend a specific duration for repeated exposure, but it included trials with an intervention of three consecutive days or more for evaluation of the effect of different carbohydrates on outcomes such as satiety and energy intake (43).

Based on these considerations, the main aim of this two-arm, randomised, crossover study was to examine the impact of sustained exposure, over 7 days, to jasmine rice with and without LAGG added during cooking on post prandial glycaemic response. The hypothesis to be tested was that the difference in response between the rice with and without LAGG added would be sustained over the 7 days. The primary objective was thus to test the sustained impact of LAGG on the primary outcome measure, the iAUC for blood glucose, using the finger prick

techniques. It was also proposed that the addition of LAGG to a rice meal would have a sustained effect on any acute changes in appetite. Exploration of the pattern, and sustainability of any impacts of adding LAGG to jasmine on appetite measured both acutely and during the 7 day intervention period, was thus the secondary objective. The following secondary outcome measures were considered following consumption of a rice test meal and over the 7 days of the intervention period: subjective appetite response measured using visual analogue scores and estimation of total energy intake over the day.

5.2 Methods:

5.2.1 Study design, Ethics and randomisation

The study took place at the SPMIC at the UoN between June 2022 and November 2022. Informed written consent was obtained from each participant before the trial. A CRF was kept according to good clinical practice. The study was approved by the UoN Faculty of Medical and Health Sciences Research Ethics Committee (Ethics approval number 414-1121) and it was registered on clinicaltrials.gov with Identifier NCT05713227.

This study was a randomised, controlled, cross-over trial. The randomisation was carried out by the study investigator using a simple Latin square design in order to minimise order effects. Allocation to the intervention was carried out following the Latin square design with participants allocated in a consecutive sequence matching prospective

recruitment numbers to avoid allocation bias. There was no selection bias and participants were recruited consecutively if they matched the eligibility criteria at screening.

5.2.2 Eligibility

Inclusion criteria:

- Aged between 18 and 65 years old.
- Able to give informed consent.
- BMI ≥18.5 and ≤24.9 kg/m²
- Apparently healthy: no medical conditions or previous gastrointestinal surgery which could affect study measurements.

Exclusion criteria

A participant was not eligible for the study if any of the following applied:

- Fasting finger prick screening blood glucose level higher than 5.4 mmol/L.
- Restrained eating behaviour as determined by SCOFF screening questionnaires (186) (shown in Appendix 8.5).
- Strenuous exercise for more than 10 hours per week
- Reported weight loss or gain ≥ 10 % of bodyweight during the six months period before the pre-study examination.
- Following a medically- or self-prescribed diet during the two weeks prior to the pre-study examination and until the end of the study

- Dislike of the products served as the dietary test treatments.
- Any allergy or food intolerance to the test treatments
- Pregnancy.
- Poor understanding of the spoken and/or written English language
- Having taken part in a research study in the last 3 months involving invasive procedures or an inconvenience allowance

5.2.3 Recruitment

Participants were recruited via posters placed around the UoN. Those expressing an interest were sent the PIS to read and if interested, they were asked to attend a short appointment for 30 minutes at the study unit when informed written consent was obtained (Appendix 8.9) and then screening was undertaken to determine if they were eligible for the study. Potential participants were asked to fast for at least 11 to 12 hours prior to the appointment. Screening included a finger prick blood sample to test for fasting blood glucose level. This was carried out using the same methods, hand-held blood glucose meter and test strips as indicated in (Section 3.2.6.1). Anthropometric data were also collected, including weight and height to calculate the BMI as defined in section 1.1. The participants filled in , a General health checklist (Appendix 8.4), and a SCOFF screening questionnaire (186) (Appendix 8.5). Suitable participants were then requested to complete a 3-day food intake record (Appendices 8.1 and 8.2) as an estimate of their habitual intake, having

been trained. They were required to bring this to the first test day. Twelve participants were screened, of which 8 were randomised and completed the study as shown by the study CONSORT flow diagram (Figure 5.1).

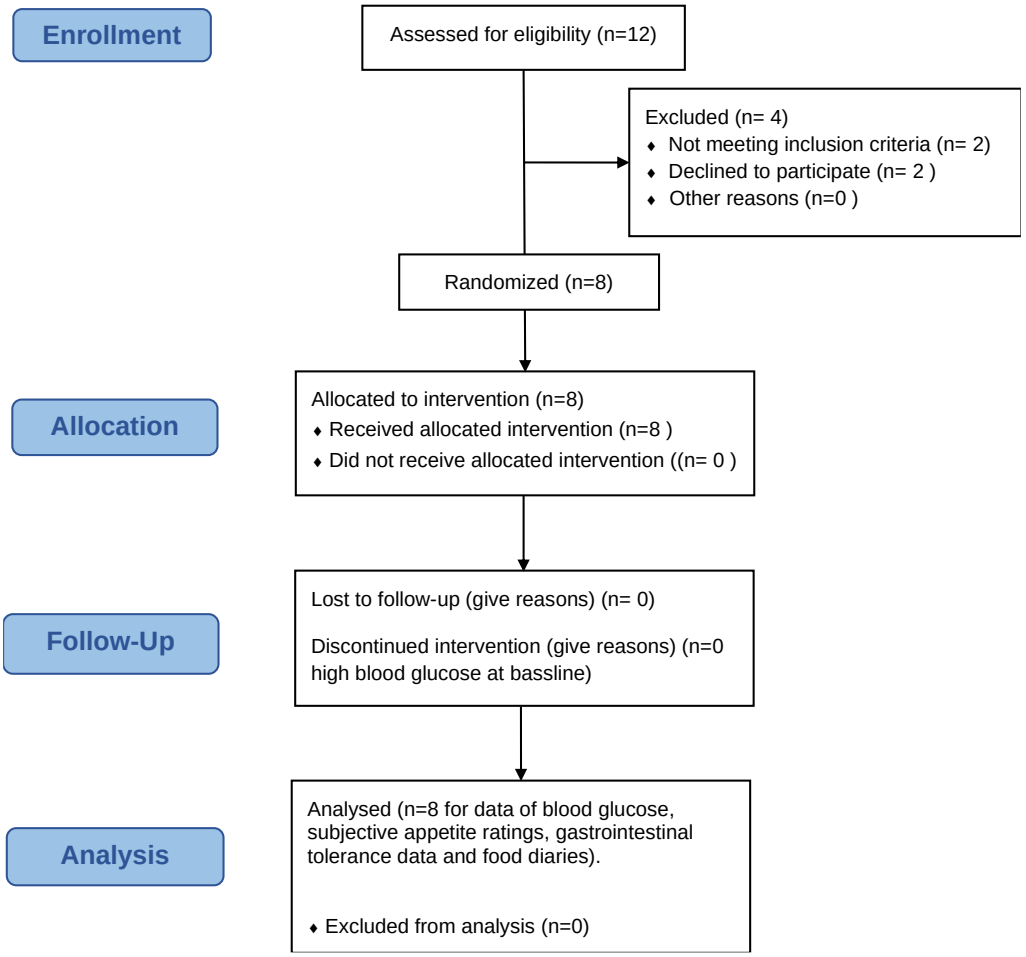


Figure 5.1 CONSORT 2010 Flow Diagram (187).

5.2.4 Study protocol and procedures during the pre and post intervention study day and 7 day intervention

Crossover trial design

The participants attended for a study day visit, followed the intervention for 7 days whilst otherwise free living and then attended for a second study day. Following a three-week washout period they then repeated the protocol but adhering to the opposite intervention during the 7 days between study days. Figure 5.2 shows the crossover trial design. The participants consumed their habitual diet between each visit and were asked to eat the same evening meal provided by the study investigators before each study day.

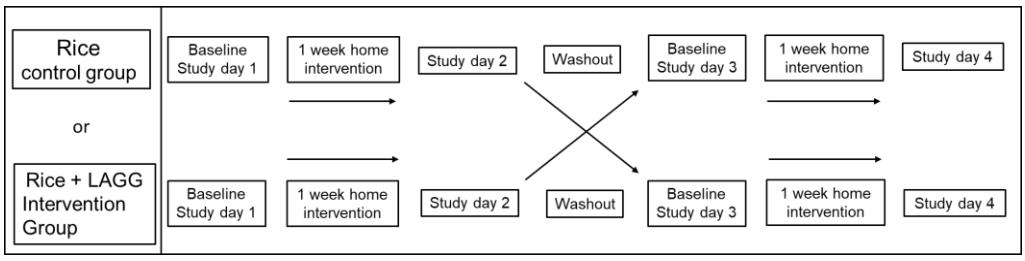


Figure 5.2 Schematic design of the crossover trial. The washout period for this study was three weeks.

Study day

The participants arrived at 09:00 hours after having fasted since 21:00 hours the previous evening (having consumed a standard meal) and having avoided alcohol, strenuous exercise, and caffeine for the 24 hours prior. Participants filled out a study eligibility check specific to this study (Appendix 8.10) at the start of each study day to ensure they had adhered to the instructions.

A schematic diagram of the timings of the study day's protocol is shown in Figure 5.3. Before the test meal, termed as time T=0 throughout, a fasting finger prick blood test was carried out using the same equipment as previously described (Section 2.2). This test provided the baseline glucose level and also a check that the fasting glucose on the study day remained in the eligible range. The participants then filled baseline VAS for subjective appetite and gastrointestinal symptoms (Section 2.3).

After this, the participants were asked to consume the test meal within 15 minutes. The gum is tasteless and both rice meals were provided in exactly the same way to ensure blinding. The rice was provided with 330 mL of still water and the participants were asked to consume all of the water with their meal.

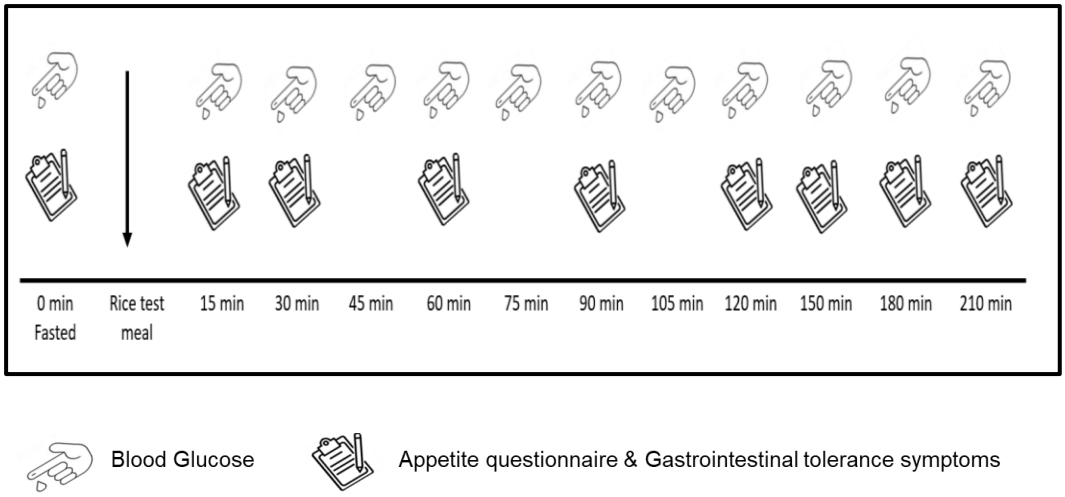


Figure 5.3 Schematic diagram of the events during the human study day.

Postprandially, fingerpick blood glucose sampling was then carried out every 15 minutes for 3.5 hours (total of 12 sampling points including fasting baseline).

VAS were completed then after feeding (T=15 min) and subsequently at 30 minutes intervals for 3.5 hours after blood glucose sampling. The gastrointestinal tolerance symptoms questionnaire was also collected at baseline and after feeding (T=15 min) and subsequently at 30 minutes intervals for 3.5 hours.

At the end of the day participants were provided with a food diary and requested to complete it for the remainder of the day in order to calculate the total energy intake for the study day.

Instructions for the intervention period

Once all activities had been completed on the study day at the start of the intervention period, participants were given a step count pedometer and with instructions about how to record the step count. They were also asked to keep a 7 day food intake diary during the dietary intervention period. They were then given 7 pre-weighted rice portions as required by the arm of the study they were completing, a rice cooker and given the cooking instructions listed in Section 2.1. They were also trained on site with respect to the cooking procedures. They were instructed to incorporate the rice into their evening meal and otherwise eat *ad libitum*.

Participants were requested to complete VAS for subjective appetite, and gastrointestinal symptom scores on waking and just prior to going to bed on the intervention days.

After a washout of 3 weeks the participants came back for the second arm of the study.

At the end of their last visit, the participants were asked 3 yes/no standard questions: if the study was acceptable, if the meals were acceptable and if they perceived a difference between the two meals.

5.2.5 Dietary standardisation pre intervention, the test meal and dietary intervention

5.2.5.1 Standardization of food before the study day

Participants were instructed to consume the same self-selected menu 24 hours prior to the study day with the exception of their final meal which they were provided with. They were requested to consume the standard evening meal the night before by 9:00 pm. This consisted of a 392g ASDA Vegetable Chilli, 250 g Tilda Microwave Pure Basmati Rice, 113 g Dole Fruit Salad. Table 5.1 shows the total energy, protein, fat and carbohydrate contents of the products which are illustrated in Figure 5.4.

They were instructed to consume as much as they required but maintain the proportions of the food items if they did not consume everything.

Figure 5.4 The standardised evening meal before the study day consisted of 392g ASDA Vegetable Chilli, 250 g Tilda Microwave Pure Basmati Rice and 113 g Dole Fruit Salad Fruit Snack.

Table 5.1 Nutritional value for the pre -study day meal.

Food item	Energy /100 g (/item)	Fat	Carbohydrates	Protein
ASDA Vegetable Chilli 392 g	73kcal (286)	2.3 g (9)	9 g (35)	2.5 (9.8)
Tilda Microwave Pure Basmati Rice 250g	143kcal (357)	1.9 g (4.75)	28.2 g (70)	2.9 g (7)
Dole Fruit Salad Fruit Snack (113 g)	57 kcal (64)	0	13 g (14)	0
Total per meal provided	707 kcal	13.75g	119 g	16.8 g

5.2.5.2 Rice meals and rice to be consumed during the intervention

The rice consumed on the study days comprised the two test meals based on jasmine rice cooked in water with and without the 3% LAGG rice meal as described before. Both test meals were prepared and served to the participants following the same method as mentioned in section 4.2.2 and provided 232 kcal for each meal at the pre and post intervention study days.

The participants were given a set of 7 individual portions of rice meal for one study arm to be cooked and consumed once /day in the evening. For the other study arm they were given a set of 7 individual portions of rice meal and a set of 7 portions of 5.5 grams of LAGG to be cooked with

the rice (Figure 5.5). They were instructed to incorporate the rice into their evening meal, and otherwise eat *ad libitum.*

Figure 5.5 Uncooked test meal portions given to participants for the cooking procedures at home.

A rice cooker (Cookworks 1.5L Rice Cooker, Argos,UK) and kitchen scale (Salter Electronic Scale with Steel Platform, Argos, UK) were also given to the participants to take home (Figure 5.6). Each participant was provided with explanations and instructions on how to cook the rice meals as described in Section 2.1.

Figure 5.6 Rice cooker (Cookworks 1.5L Rice Cooker, Argos,UK) and kitchen scale (Salter Electronic Scale with Steel Platform, Argos, UK) given to the participants to take home.

5.2.6 Outcomes

5.2.6.1 Blood glucose

The blood glucose responses were measured using the same finger prick methods and equipment as described in Section 2.2.

5.2.6.2 Subjective appetite responses

Paper-based 100 mm VAS were used to measure the subjective feeling of hunger, satiety, fullness, desire to eat and PFC, following the same method as described in Section 2.3.

5.2.6.3 Gastrointestinal tolerance

VAS were used to measure gastrointestinal symptoms of Gas/ Flatulence, Bloating, Abdominal Pain and Diarrhoea as described in the methods Chapter (Section 2.3.5).

5.2.6.4 Food diaries

Habitual food diaries

Participant were requested to a complete food diary (as described in Section 2.3.5) for 3 days before the start of the 7-day trial. The investigator met with the participants and clarified with the participants entries where necessary. The diaries were again analysed using Nutritics software (Nutritics Ltd, Dublin, Ireland).

Measurements of food intake during the 7-day trial

Participants were given a study booklet including a food diary and requested to record total food intake daily for the 7 days period. They were asked to keep a detailed record of food and beverages consumed over the 7-days. In keeping with the previous study, food records and analysis followed again the methods described in Section 2.3.5.

5.2.6.5 Activity monitoring during the study trial

Participants were provided with a pedometer (GRV Pedometer Watch, China, Figure 5.7). They were requested to wear it continuously during

the 7 days trial period, unless showering or swimming, to record the daily step counts. At the end of each day, once in bed they were asked to write down in a study record form the step count shown in the pedometer.

Figure 5.7 Image of the GRV Pedometer Watch fitness tracker provided to each participant.

A summary of the interventions and outcomes recorded during the 7-day study is shown in Figure 5.8.

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Food Diary	√	√	√	√	√	√	√
Step count	√	√	√	√	√	√	√
180 g evening Rice Test meal	√	√	√	√	√	√	√
Subjective appetite responses (am &pm)	√	√	√	√	√	√	√
Gastrointestinal tolerance (evening)	√	√	√	√	√	√	√

Figure 5.8 A summary Intervention and outcomes recorded for the 7-days study.

5.2.7 Data and statistical analysis

Sample size calculation

The sample size was calculated based on the pilot data from the study shown in Chapter 4 as it used the same two meals as in this current study. Those data showed a change in the standard 2-hours postprandial iAUC for blood glucose with Delta = difference in population means of (mean rice iAUC – mean rice+LAGG iAUC) = 68.4 mmol/L min and SD (standard deviation of difference in the iAUC response of matched pairs calculated from the data) = 50.2 mmol/L min. Based on these data it was possible to predict that to observe that effect size in a paired cross-over study with $P < 0.05$ two tailed and 90% power one would require N=8 participants as determined using the program PS Power and Sample Size (Vanderbilt University, TN, USA).

Data presentation and analysis

Descriptive data were presented as means with the SEM unless stated otherwise.

The blood glucose data was collected on hard copy CRF then transcribed to a Microsoft Excel sheet. The iAUC 2h was calculated for blood glucose for each participant and arm of the study. The iAUC for the whole duration of the study day was also calculated and is defined here as iAUC 210 min. For all the other data, the total AUC 2h was calculated from the respective time curves for each participant and arm of the study. The

Shapiro-Wilk normality was used to test for normal distributions. This confirmed a paired t-test was appropriate to compare AUCs for most data sets. The data were expressed as means with SEM.

The data was analysed using GraphPad Prism for windows version 9.2.0 (GraphPad Software, San Diego, California). Statistical significance of differences was assumed with a P value less than 0.05.

5.3 Results:

Eight participants completed the human study, five female and three male. They were (mean ± SEM) 36 ± 4 years old, with a BMI of 23 ± 1 kg/m², a weight of 67 ± 3 kg and height of 170 ± 3 cm, Demographic data for the study participants are shown in Table 5.2.

The study procedures were tolerated well by all participants. All participants consumed the rice meals allocated at the study unit within the time required and they did not report any problem in preparing and consuming the meals at home. There were no adverse events reported during the study. Eight complete data sets were available for analysis for all the study outcomes.

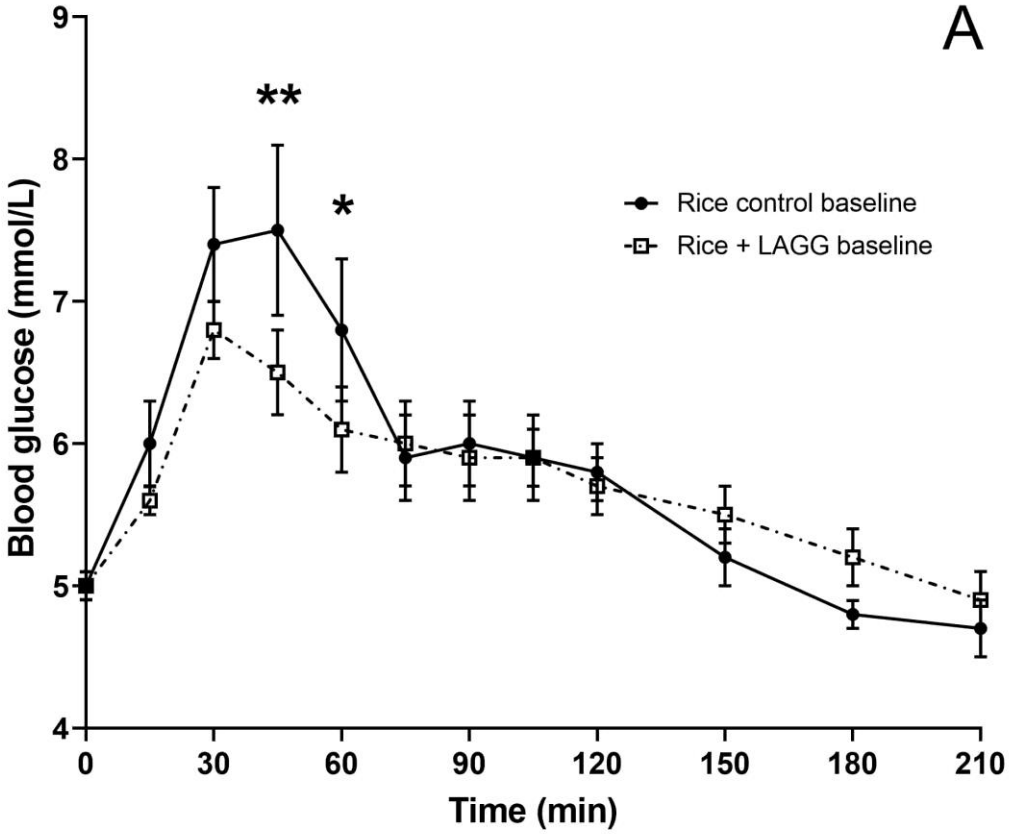
Table 5.2 Demographic data by sex for the study participants.

	Male n=3	Female n=5
Age (years)	42± 9	33 ± 3
Weight (kg)	74± 0	64 ± 3
Height (cm)	178± 1	167 ± 2
BMI (kg/m^2)	23± 0	23 ± 1

5.3.1 Blood glucose

The blood glucose time courses are shown in Figure 5.9 A and B. The baseline values were within the healthy normal range and there was no baseline difference between arms of the study, being 5.0 ± 0.1 mmol/L for the rice control meal both before and after the week of intervention and 5.0 ± 0.1 mmol/L for the rice + LAGG meal before and 4.9 ± 0.1 mmol/L after the week of intervention ($P > 0.9$).

Blood glucose rose rapidly after feeding the rice meals within the first half and hour and then declined slowly. The graph in Figure 5.9 A and B have similar features to those seen in Chapter 3 for the previous study, in that the blood glucose values for the rice control meal rose higher than the values for the rice + LAGG intervention.


A
**
*
Rice control baseline
Rice + LAGG baseline
Blood glucose (mmol/L)
9
8
7
6
5
4
0
30
60
90
120
150
180
210
Time (min)

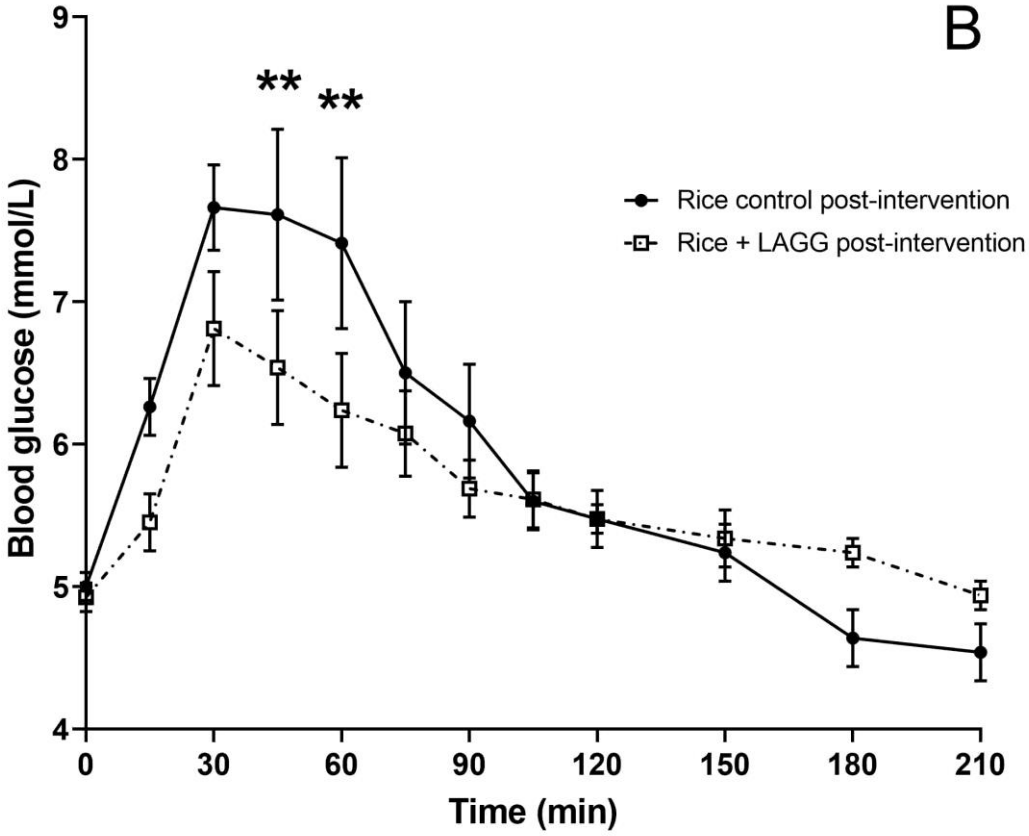

B
**
**
Rice control post-intervention
Rice + LAGG post-intervention
Blood glucose (mmol/L)
9
8
7
6
5
4
0
30
60
90
120
150
180
210
Time (min)

Figure 5.9 Blood glucose time courses from N=8 healthy participants who consumed the rice meal with and without the addition of low acyl gellan gum (LAGG) during the cooking process. The time courses are shown both for (A) the study day at baseline and (B) for the study day after 1 week of repeated intervention (termed post-intervention). ANOVA analysis over the whole time course showed a significant interaction of rice meal type × time both for plots A at baseline (P = 0.0023) and for plots B after 1 week intervention (P = 0.0440). Data points are mean ± SEM. * $P < 0.05$ and ** $P < 0.01$.

The iAUC 2h for blood glucose is shown in Figure 5.10. Comparing the rice control and rice + LAGG for baseline (acute) intervention, the iAUC 2h was 21% lower for rice + LAGG meal compared with the rice control meal but the difference was not significant with (P = 0.1812), shown in Table 5.3. Over the first 2 hours postprandially the individual blood glucose levels rarely went below baseline and therefore the iAUC 2h calculation (baseline corrected and ignoring negative areas below baseline) are virtually unchanged when using a simple AUC 2h trapezoidal integral (total AUC with no baseline correction).

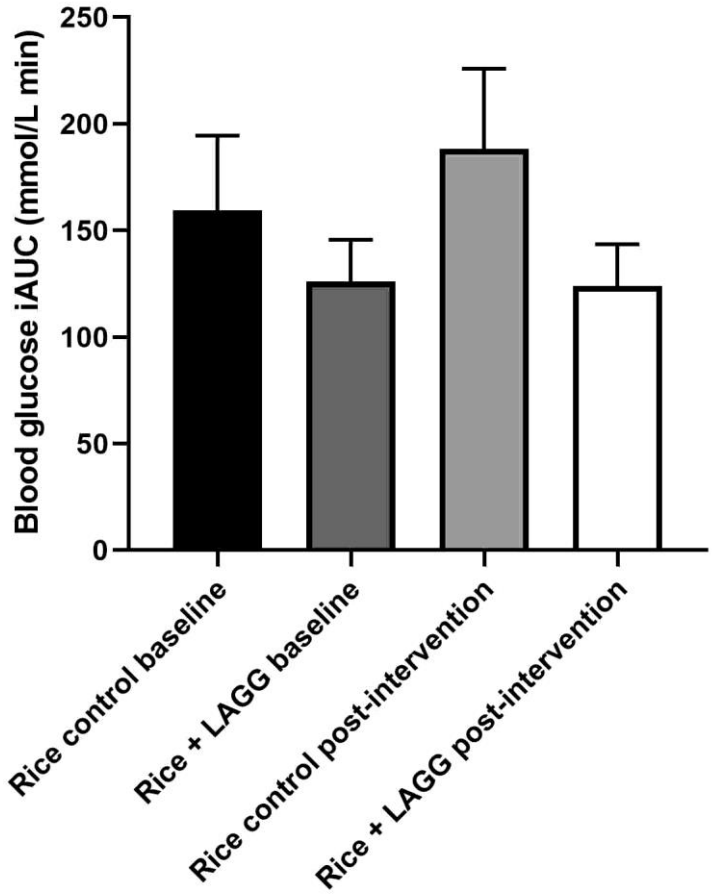


Figure 5.10 Blood glucose iAUC2h from N=8 healthy participants who consumed the rice meal with and without the addition of low acyl gellan gum (LAGG) during the cooking process. The iAUCs are shown both for the study day at baseline and for the study day after 1 week of repeated intervention (termed post-intervention). Data points are mean ± SEM.

Looking at Table 5.3, interestingly the rice + LAGG meal did not change the blood glucose iAUC 2h after 1 week of sustained intervention, but the iAUC 2h increased by 16% for the rice control meal after 1 week of sustained intervention though this difference was not significant with P = 0.3051. The iAUCs 210 minutes results are similar to those for iAUC 2h.

Two-way repeated measure ANOVA analysis over the whole time course showed a significant interaction of rice meal type × time both at baseline (P = 0.0023) and after 1 week intervention (P = 0.0010). Following this, Sidak's *post-hoc* multiple comparison test showed significant difference between blood glucose means for rice control and rice + LAGG, at baseline, at time T=45 min (P = 0.0014) and T=60 min (P = 0.0440). Similarly, the *post-hoc* multiple comparison test showed significant difference between blood glucose means for rice control and rice + LAGG, after 1 week intervention, at time T=45 min (P = 0.0039) and T=60 min (P = 0.0021).

Blood glucose rose postprandially to a peak at T=30 minutes. Peak values are shown in Figure 5.11. The rice peak 7.7 ± 0.5 mmol/L, was higher than for the rice + LAGG meal 7.0 ± 0.2 mmol/L though this difference was not significant (P = 0.1720). Interestingly the peak blood glucose value for the rice control meal rose to 8.2 ± 0.5 mmol/L for the rice control meal after 1 week of intervention and the mean value was 1.2 mmol/L higher than that for the blood glucose peak value mean for the rice + LAGG meal after 1 week of intervention (P = 0.0219, corrected for multiple comparisons).

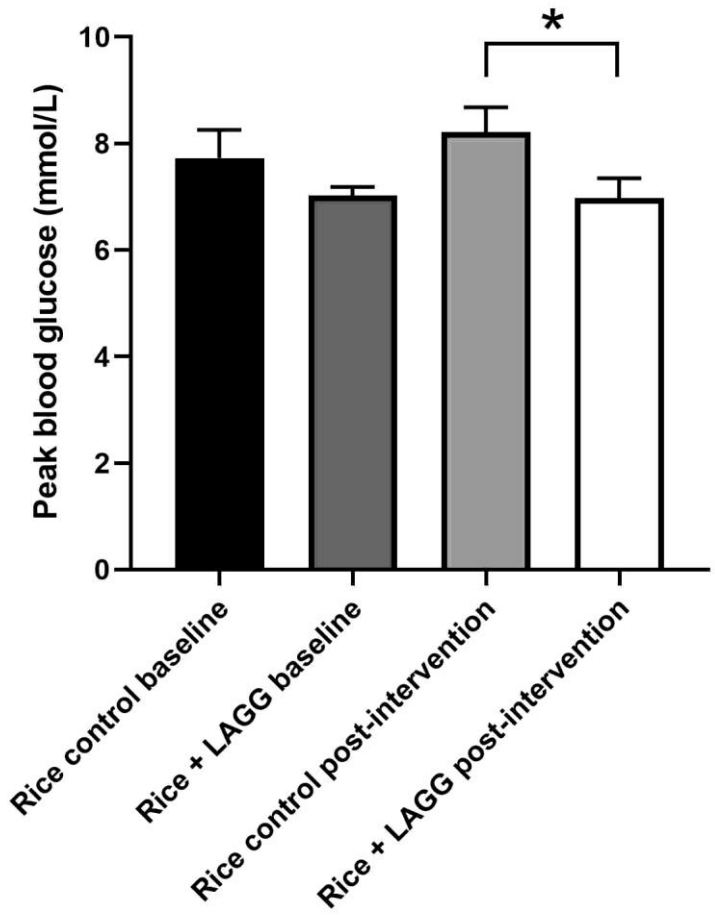


Figure 5.11 Peak blood glucose value from N=8 healthy participants who consumed the rice meal with and without the addition of low acyl gellan gum (LAGG) during the cooking process. The peak values are shown both for the study day at baseline and for the study day after 1 week of repeated intervention (termed post-intervention). Data points are mean ± SEM. * *P* <0.05.

The period of postprandial glucose measurement was longer than that in Chapter 3 and allowed consideration of the time to return to baseline (TTRTB) of the blood glucose values. This is best seen when baseline correcting the graph at Figure 5.12 so that the baseline value was shifted

to 0 mmol/L for each participant. Blood glucose values gradually decreased and by T=120 minutes had not yet gone back to the baseline values. The values for the rice + LAGG meal remained consistently lower than those for the rice control meal. Across the whole study the mean of the individual blood glucose values for the rice + LAGG meal were more than 1 mmol/L lower than for the corresponding rice control meal. Overall, in Figure 5.12 it can be seen that the rice meal with LAGG delayed the mean glucose excursion's TTRTB by 50 minutes.

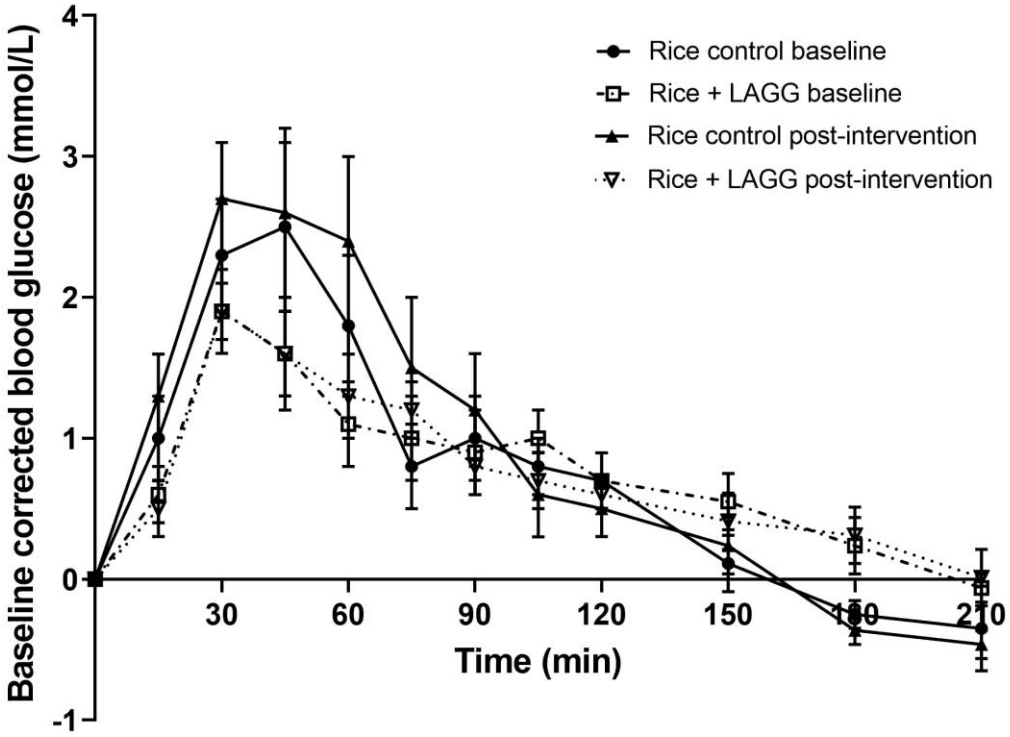


Figure 5.12 Baseline corrected blood glucose time courses from N=8 healthy participants who consumed the rice meal with and without the addition of low acyl gellan gum (LAGG) during the cooking process. The baseline corrected time courses are shown both for the study day at baseline and for the study day after 1 week of repeated intervention (termed post-intervention). Data points are mean ± SEM.

Table 5.3 Summary results table for N=8 participants blood glucose, appetite and food record data who consumed the test meal with and without the addition of low acyl gellan gum (LAGG) collected on the study day visits. Data are shown as mean ±standard error of the mean. The P values in the last column were t tests of Rice control versus Rice + LAGG.

	Rice control	**Rice + LAGG**	***P value***
Baseline glucose iAUC 2h (mmol/L·min)	159 ± 35	126 ± 20	0.1812
Post-intervention glucose iAUC 2h (mmol/L·min)	188 ± 38	124 ± 19	0.1209
Baseline glucose iAUC 210 min (mmol/L·min)	180 ± 38	163 ± 29	0.5500
Post-intervention glucose iAUC 210 min (mmol/L·min)	208 ± 38	159 ± 19	0.2997
Baseline peak blood glucose (mmol/L)	7.7 ± 0.5	7.0 ± 0.2	0.1720
Post-intervention peak blood glucose (mmol/L)	8.2 ± 0.5	7.0 ± 0.4	0.0219
Baseline composite appetite score AUC 210 min (mm·min)	11978 ± 1200	13489 ± 1000	0.2861
Post-intervention composite appetite score AUC 210 min (mm·min)	14099 ± 630	14797 ± 566	0.1644

Baseline hunger AUC 2h (mm·min)	9994 ± 1435	12321 ± 1380	0.1576
Post-intervention hunger AUC 2h (mm·min)	12205 ± 902	13071 ± 1092	0.1953
Baseline satisfaction AUC 2h (mm·min)	7418 ± 1061	6469 ± 1105	0.5012
Post-intervention satisfaction AUC 2h (mm·min)	6006 ± 859	5348 ± 561	0.3497
Baseline fullness AUC 2h (mm·min)	7400 ± 1208	5991 ± 1110	0.2460
Post-intervention fullness AUC 2h (mm·min)	5403 ± 860	4868 ± 685	0.4084
Baseline desire to eat AUC 2h (mm·min)	10530 ± 1350	12428 ± 1204	0.2709
Post-intervention desire to eat AUC 2h (mm·min)	13447± 768	14441 ± 485	0.1013
Baseline prospective consumption AUC 2h (mm·min)	12183 ± 1365	13157 ± 677	0.4954
Post-intervention prospective consumption AUC 2h (mm·min)	14253 ± 529	14689 ± 669	0.4387
Average self-reported daily energy intake for the 7 days of intervention (kcal)	1502 ± 62	1472 ± 93	0.6085
Average steps count / day	9507 ± 669	9965 ± 622	0.2188

5.3.2 Subjective appetite responses during the study day

Figures 5.13 to 5.18 show the time courses for individual and composite subjective appetite responses to the two rice test meals. Considering hunger, desire to eat and prospective consumption, the decrease from baseline was numerically higher for the rice control compared with the rice meal with LAGG. However, the differences between the areas under the curves of all the appetite rating scores between rice control meal and rice + LAGG meal were not statistically significant (Table 5.3). For the composite appetite score the two-way repeated measure ANOVA analysis over the whole time course showed no significant interaction of rice meal type × time both at baseline (P = 0.2411) and after 1 week intervention (P = 0.2265).

The mean morning (AM) and afternoon (PM) VAS for appetite ratings over the 7 days of the two study arms are shown in Figure 5.19 and Figure 5.20.

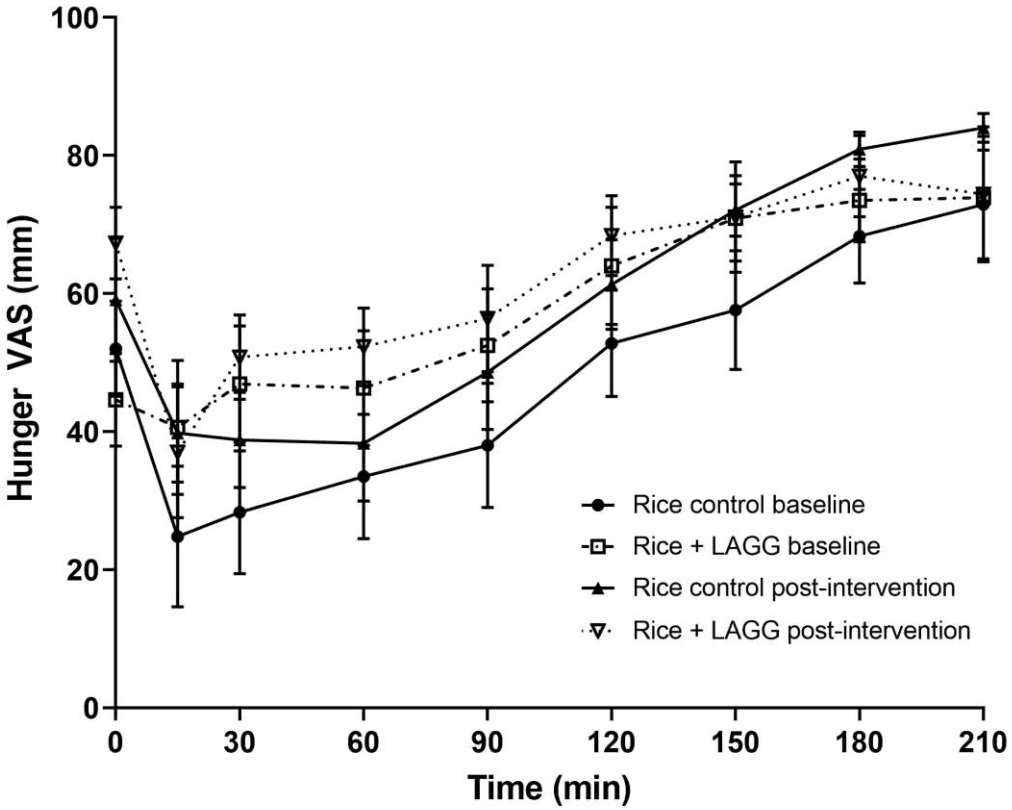


Figure 5.13 Hunger visual analogue scale (VAS) time courses from N=8 healthy participants who consumed the rice meal with and without the addition of low acyl gellan gum (LAGG) during the cooking process. The time courses are shown both for the study day at baseline and for the study day after 1 week of repeated intervention (termed post-intervention). Data points are mean ± SEM.

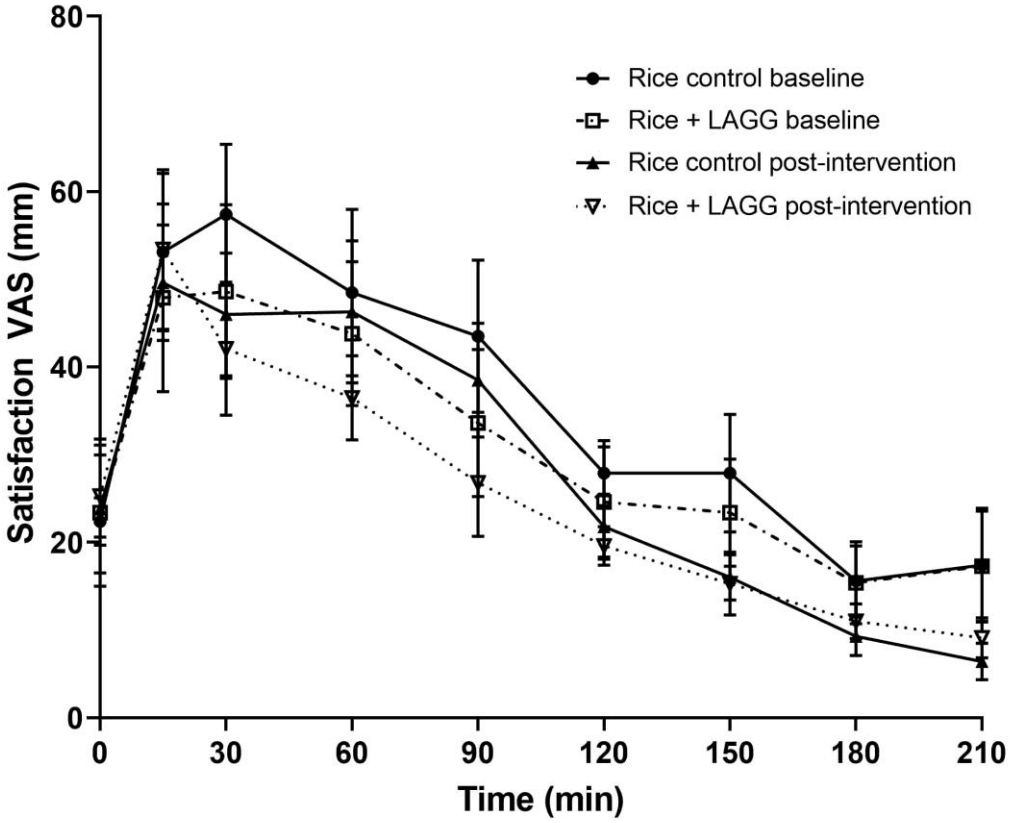


Figure 5.14 Satisfaction visual analogue scale (VAS) time courses from N=8 healthy participants who consumed the rice meal with and without the addition of low acyl gellan gum (LAGG) during the cooking process. The time courses are shown both for the study day at baseline and for the study day after 1 week of repeated intervention (termed post-intervention). Data points are mean ± SEM.

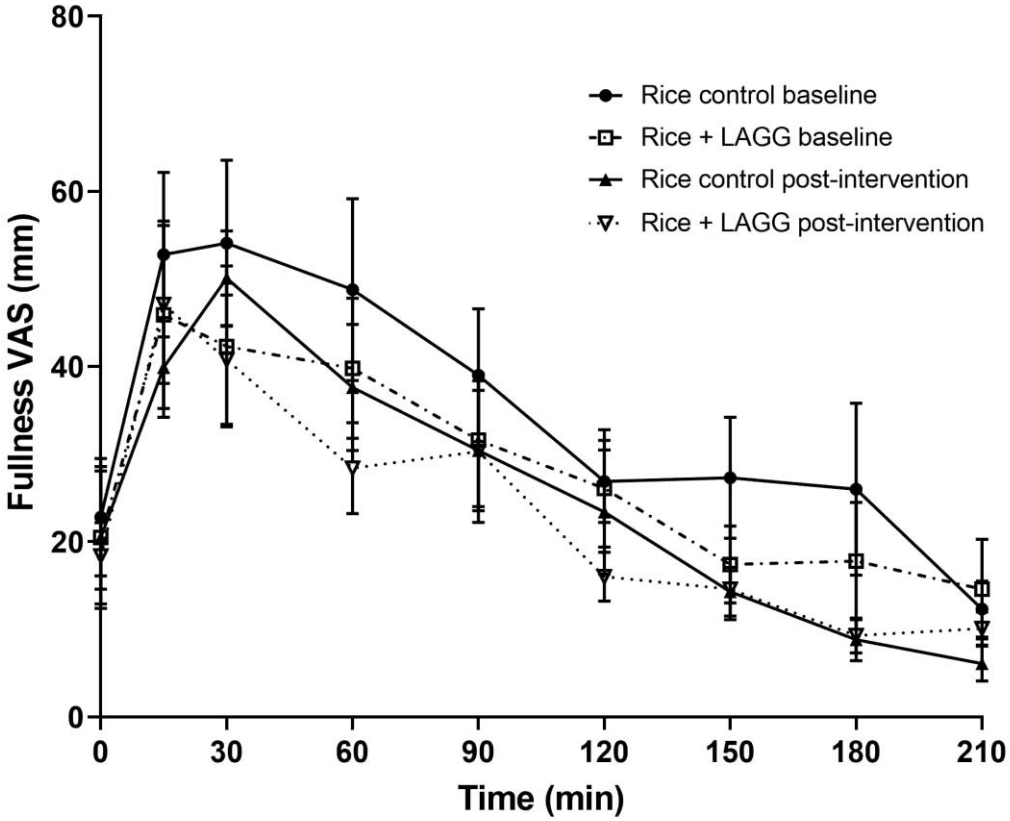


Figure 5.15 Fullness visual analogue scale (VAS) time courses from N=8 healthy participants who consumed the rice meal with and without the addition of low acyl gellan gum (LAGG) during the cooking process. The time courses are shown both for the study day at baseline and for the study day after 1 week of repeated intervention (termed post-intervention). Data points are mean ± SEM.

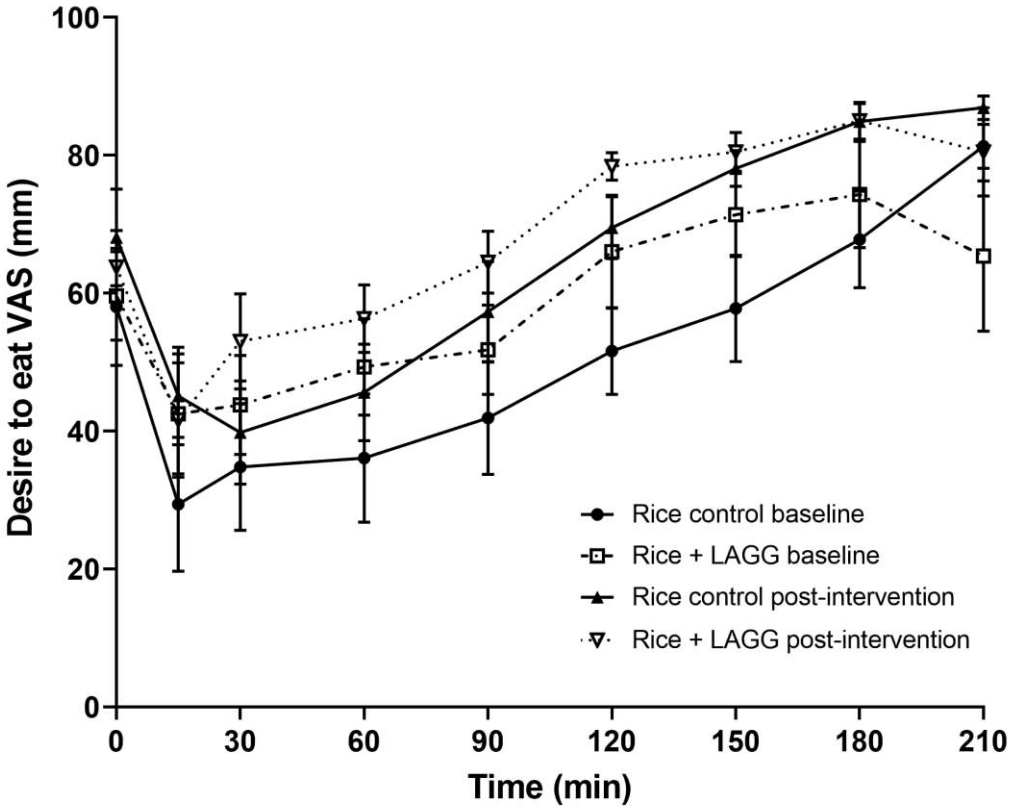


Figure 5.16 Desire to eat visual analogue scale (VAS) time courses from N=8 healthy participants who consumed the rice meal with and without the addition of low acyl gellan gum (LAGG) during the cooking process. The time courses are shown both for the study day at baseline and for the study day after 1 week of repeated intervention (termed post-intervention). Data points are mean ± SEM.

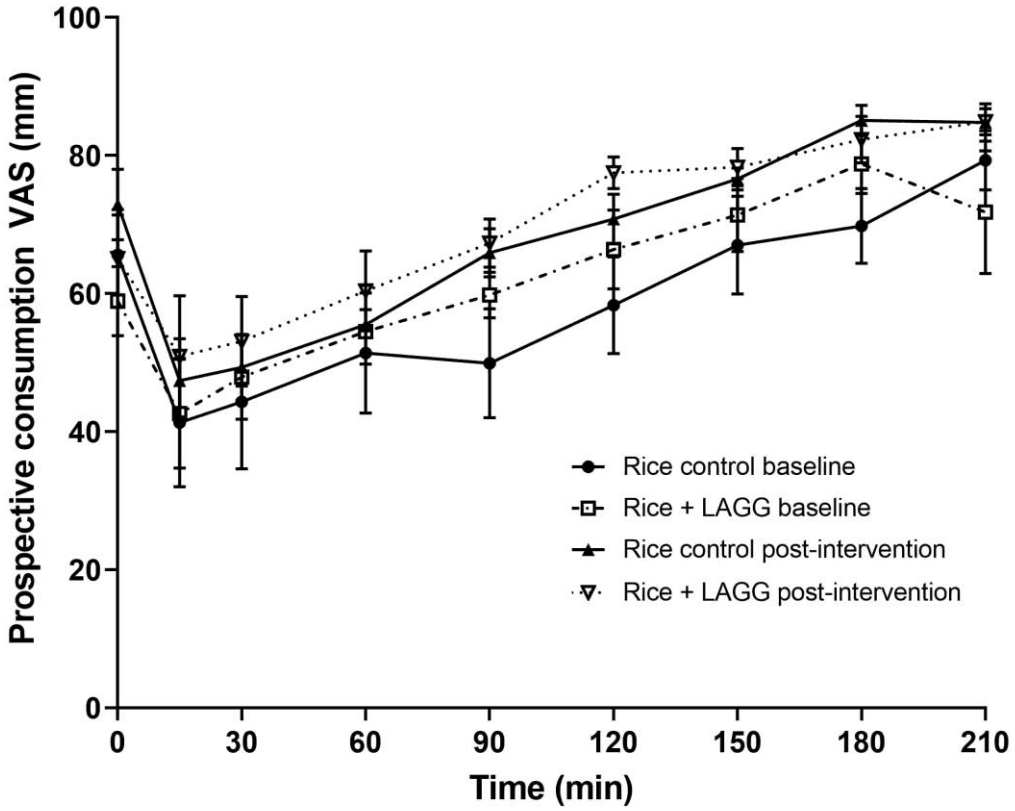


Figure 5.17 Prospective consumption to eat visual analogue scale (VAS) time courses from N=8 healthy participants who consumed the rice meal with and without the addition of low acyl gellan gum (LAGG) during the cooking process. The time courses are shown both for the study day at baseline and for the study day after 1 week of repeated intervention (termed post-intervention). Data points are mean ± SEM.

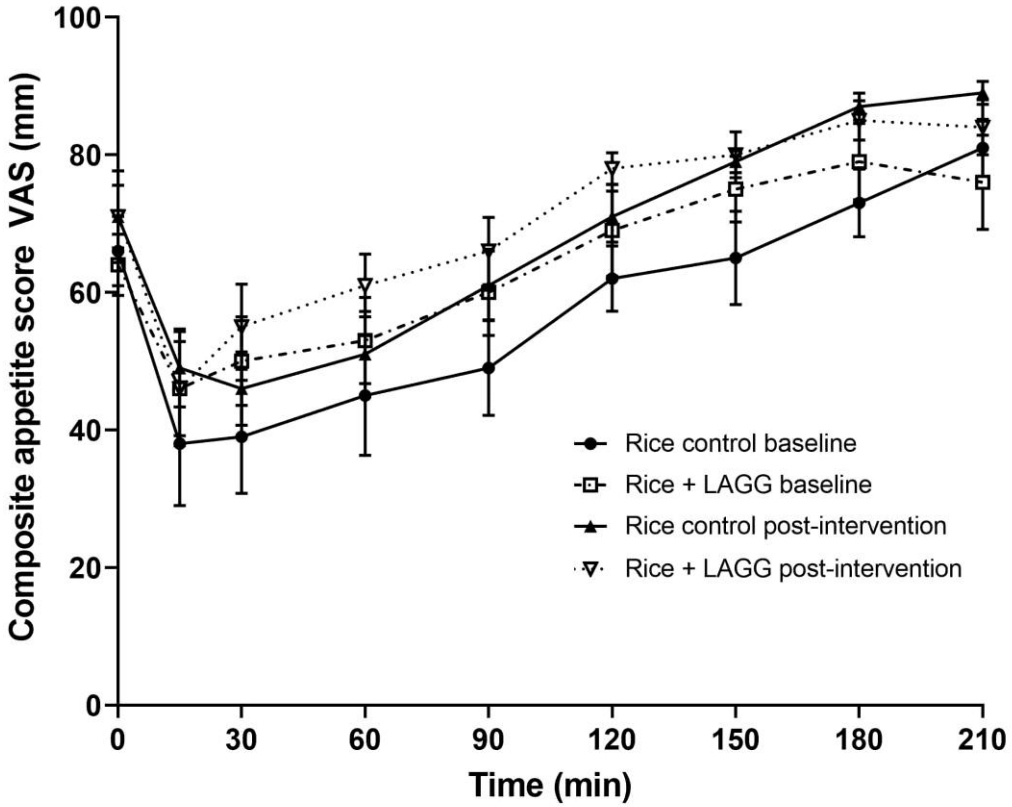


Figure 5.18 Composite appetite score to eat visual analogue scale (VAS) time courses from N=8 healthy participants who consumed the rice meal with and without the addition of low acyl gellan gum (LAGG) during the cooking process. The time courses are shown both for the study day at baseline and for the study day after 1 week of repeated intervention (termed post-intervention). Data points are mean ± SEM. Lower values in this context indicate lower hunger, higher fullness and less desire to eat, whilst higher values indicated the opposite (53, 88).

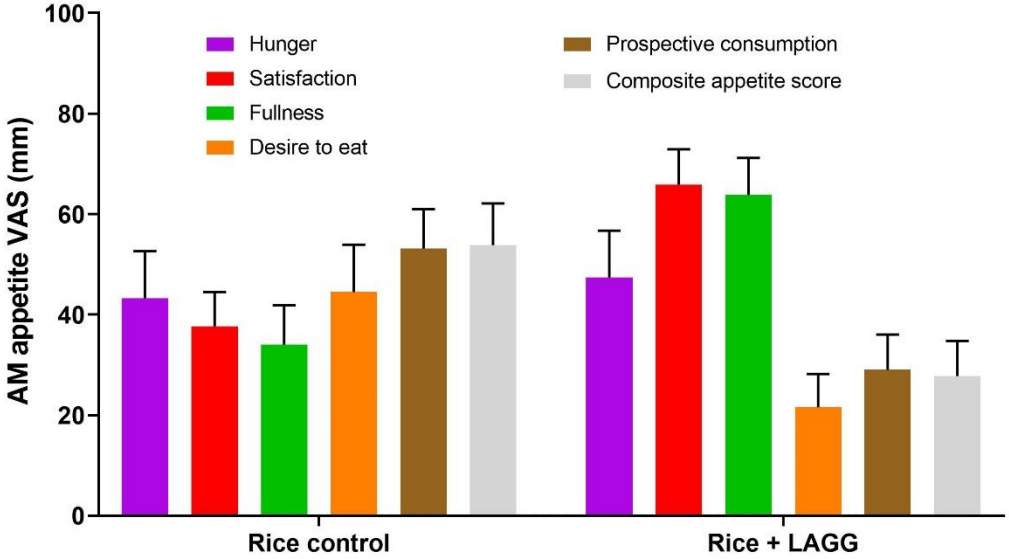


Figure 5.19 The participants recorded for each morning (AM) a visual analogue scale (VAS) of their appetite. The histogram shows the mean visual analogue scale VAS ratings for each domain averaged over the 7 days of intervention period from the N=8 healthy participants who consumed the rice meal with and without the addition of low acyl gellan gum (LAGG) during the cooking process. Data bars show the mean ± SEM.

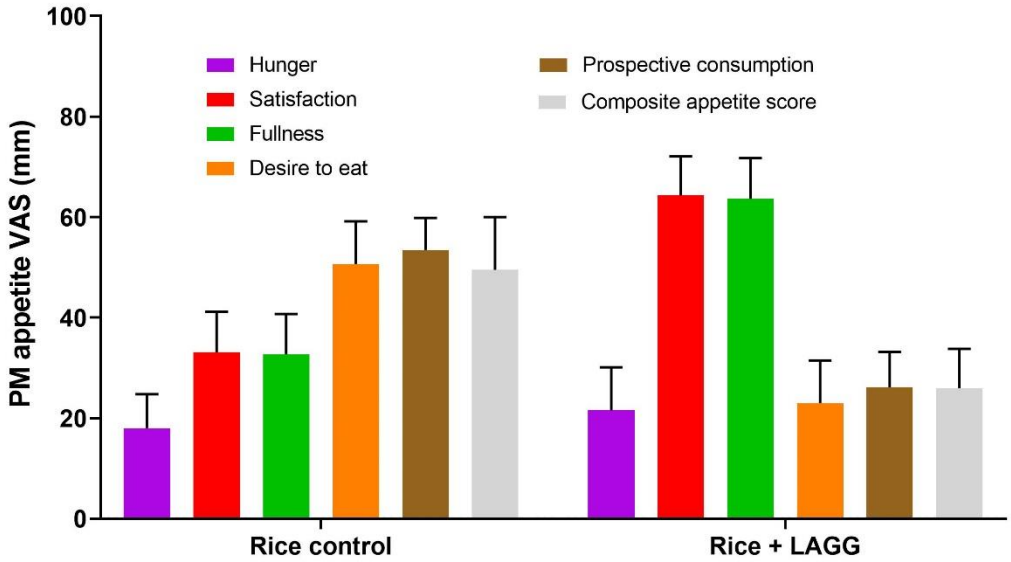


Figure 5.20 The participants recorded for each afternoon (PM) a visual analogue scale (VAS) of their appetite. The histogram shows the mean visual analogue scale VAS ratings for each domain averaged over the 7 days of intervention period from N=8 healthy participants who consumed the rice meal with and without the addition of low acyl gellan gum (LAGG) during the cooking process. Data bars show the mean ± SEM.

5.3.3 Satiety quotient

The SQ time courses for the composite appetite score are shown in Figure 5.21. The SQ time courses for the feelings of hunger, satisfaction, fullness, desire to eat and prospective consumption are shown in Appendix 8.11.

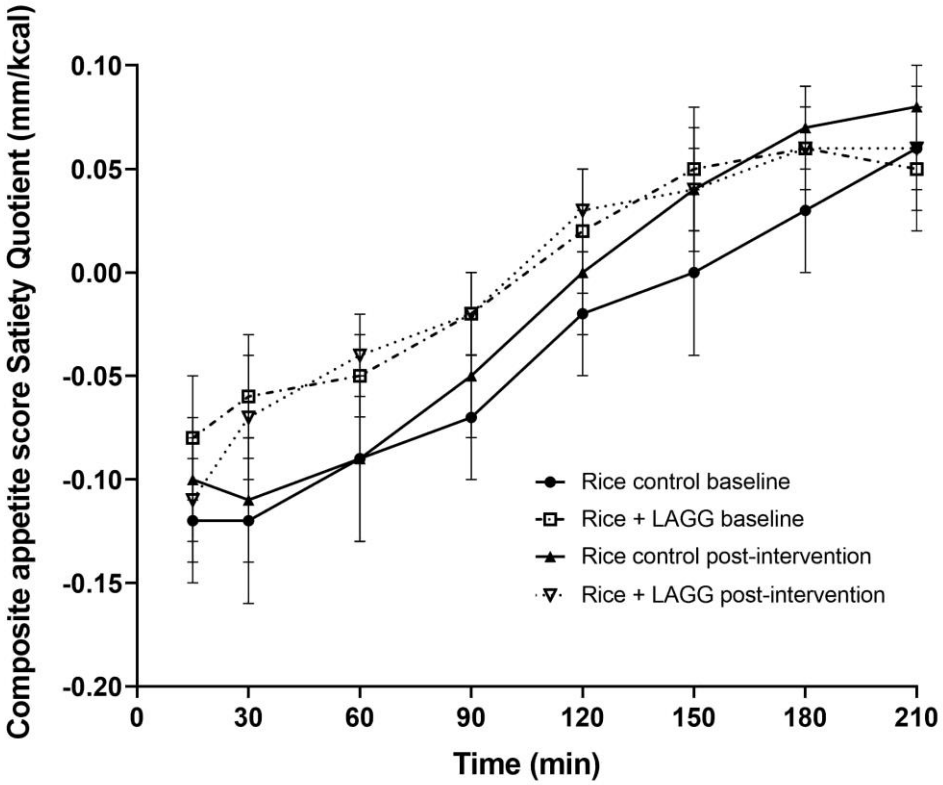


Figure 5.21 Composite appetite score Satiety Quotient time courses from N=8 healthy participants who consumed the rice meal with and without the addition of low acyl gellan gum (LAGG) during the cooking process. The time courses are shown both for the study day at baseline and for the study day after 1 week of repeated intervention (termed post-intervention). Data points are mean ± SEM.

5.3.4 Food Records during 7-days intervention

All daily food record were completed and analysed. Figure 5.22 shows the individual average (self-reported) energy intakes for the 3-days habitual diet at baseline and for the 7-days averages for both arms of the

study. The means are also shown in the graph and reported in Table 5.3. The average self-reported energy intake records during the 7 days intervention did not differ between the two arms of the study ($P = 0.6085$). The energy intake record for the day before the second blood glucose study day was also tested to check whether the participants, after a week of intervention, had a similar energy intake, prior to the rice test on the final day. There was no significant difference between energy intake on day 7 ($P = 0.5575$), prior to the test day.

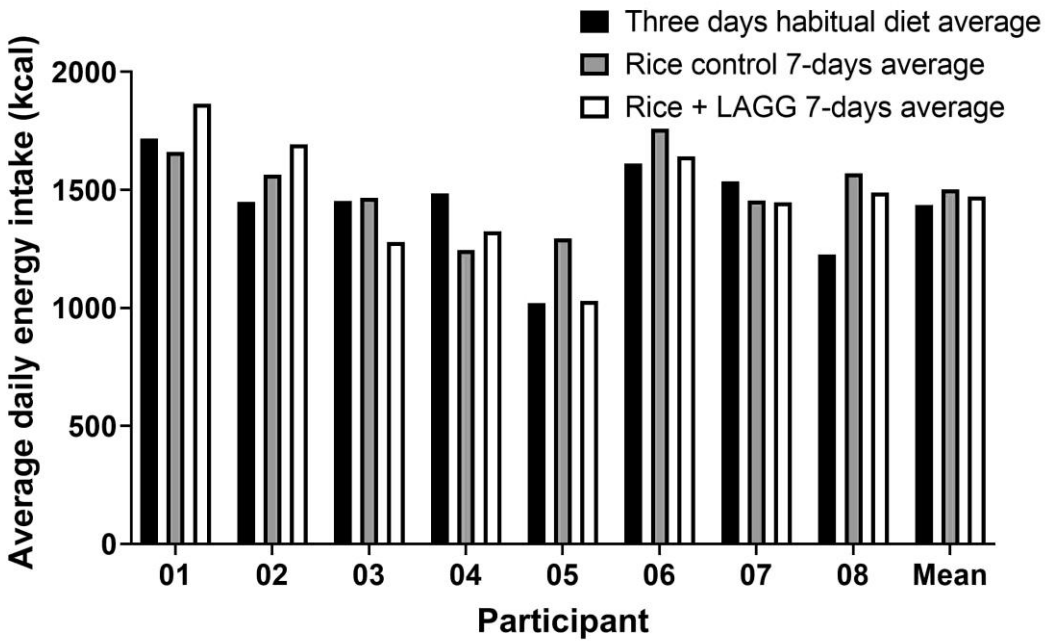


Figure 5.22 Individual average daily energy intake for each of the N=8 healthy participants who consumed the rice meal with and without the addition of low acyl gellan gum (LAGG) during the cooking process. The bar chart shows the 3 days habitual day average in black bars, the 7-days average energy intake for the rice control arm of the intervention in

grey, and the 7-days average energy intake for the rice + LAGG arm of the intervention. Data bars show the mean.

5.3.5 Gastrointestinal tolerance symptoms

The symptoms of gas, bloating, abdominal pain and diarrhoea on the study day were very low with mean VAS scales across 8 participants scoring between 0 mm and 7 mm. Figures 5.23 and 5.24 show respectively the gastrointestinal symptoms VAS ratings and the gastrointestinal symptoms score for each of the 7 days of intervention. The VAS ratings and the scores are low indicating good gastrointestinal tolerance for the intervention.

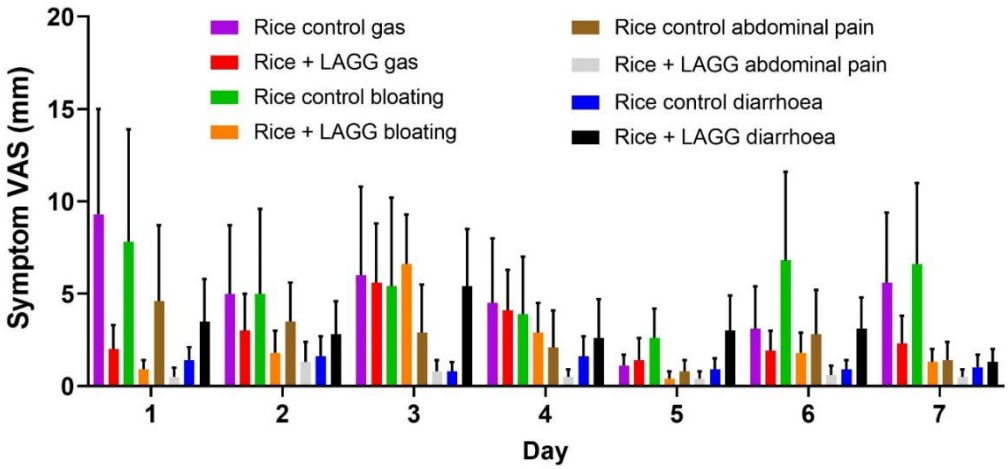


Figure 5.23 Average gastrointestinal symptoms visual analogue scale (VAS) ratings for each of the 7 days of intervention. The data are from N=8 healthy participants who consumed the rice meal with and without the addition of low acyl gellan gum (LAGG) during the cooking process. Data bars show the mean ± SEM.

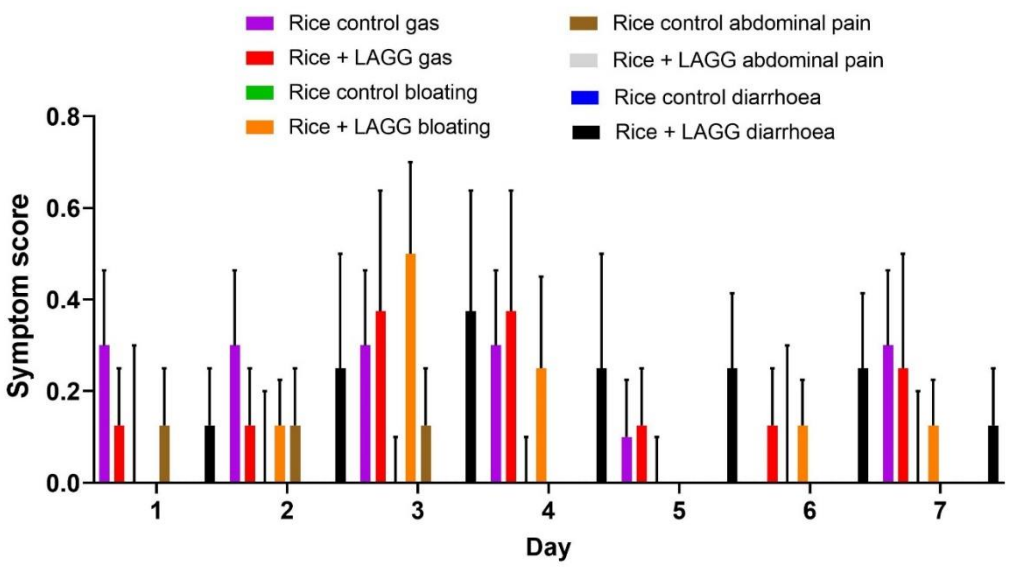


Figure 5.24 Average gastrointestinal symptoms score for each of the 7 days of intervention. The data are from N=8 healthy participants who consumed the rice meal with and without the addition of low acyl gellan gum (LAGG) during the cooking process. Data bars show the mean ± SEM.

5.3.6 Physical activity during 7-days intervention

The physical activity during the 7 days of the intervention was monitored with the pedometer. Step counts per day (Table 5.3) did not differ between rice control meal and rice + LAGG meal arms of the study (Wilcoxon's $P = 0.2188$).

5.3.7 End of study questions

At the end of their last visit, all eight participants answered yes to the question if the study was acceptable and yes to the question if the meals

were acceptable. 6 out of 8 participants said they had perceived a difference between the two rice meals.

5.4 Discussion

The rational for conducting this study was that it is not clear whether physiological and/or appetite responses measured in response to an acute, single-exposure dietary intervention would persist after the body has been exposed to the same dietary intervention repeatedly. This is because adaptation can occur in the body and this may modify the responses observed within an acute exposure study setting. Examples in the literature concern lipid absorption and the microbiota (202, 203). Repeated consumption studies are also advocated by the EFSA and the UK SACN committee (43, 205). In an expert review conducted in 2018 by the International Life Sciences Institute (ILSI Europe) and appraising literature on appetite and energy intake studies, a dietary manipulation was considered to be a chronic (sustained) exposure if its duration was equal to- or greater than- three days (204). The authors found that despite the large number of studies on appetite and energy intake only a small number of studies tested if acute effects in response to an intervention changed after chronic exposure.

The design of this study aimed to address such question and tested the blood glucose response to the rice meals at baseline and then again after one week of daily exposure to the same intervention.

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The study design was complex but worked well and all the participants returning for all the study visits as planned. Reported compliance with preparing and consuming the rice meal intervention at home was excellent.

The rice + LAGG intervention resulted in a lower blood glucose compared with the rice control during the rice test as predicted, with postprandial differences visually similar to those observed in the acute study in Chapter 4. The primary outcome, blood glucose iAUC 2h, was lower for the rice + LAGG by 21% at baseline and by 34% after 1 week intervention. However, the numerical differences were not statistically significant, most probably due to individual variability (206). The smaller sample size, although appropriately powered from the previous study, may have had A significant interaction for LAGG treatment by time was indeed observed by repeated measures ANOVA, and the effect was present both at baseline and after 1 week of the intervention.

It was interesting to note that eating a dish of white jasmine rice control per day over just 7 days was associated with an increased average peak blood glucose by 0.5 mmol/L in healthy participants and that adding LAGG to cooking significantly reduced peak glucose by 1.2 mmol/L which represents a clinically significant reduction. It has been suggested that the level of postprandial glucose peaks may be an important predictor of increased risk of cardiovascular disease in those with diabetes (207). This may explain the association seen between white rice consumption and poorer health outcomes in populations consuming more white rice.

In contrast with the acute study here appetite increased slightly with LAGG after 1 week, but the differences were not significant and there was no change in daily energy intake Interestingly, whilst subjective appetite sensations that might drive intake appear higher when rice with LAGG was given, the opposite is seen numerically in the morning and evening ratings. This might suggest that whilst the addition of LAGG to rice has decreased the satiation response to the meal, it has increased satiety, seen over the longer term (i.e. in the evening and after indeed after an overnight fast). These observations will need to be explored with a larger samples size.

No change in gastrointestinal symptoms were seen after the repeated exposure to LAGG at these doses. This was a positive finding as problems with bloating and flatulence were reported for other similar gums (208).

5.4.1 Strengths and limitations

The washout period for this study was carefully considered and set at 3 weeks. A washout period is normally required in nutrition and dietetics studies to avoid carry over effects from one period of the study to the following one (209). Due to the sustained nature of the intervention, it was felt that 1 week washout as used in Chapter 4 might have been not sufficient. A recent publication discussing effects of sustained intervention on gut microbiota showed that there is a rather variable length of washout between different studies, usually varying between 6

days to about 4 weeks (203). Based on these considerations, it was felt that 3 weeks was an adequate length.

All the questionnaires utilised here were used in various previous studies and they were all previously published.

As detailed in Chapter 4, carefully attention was paid to minimising the risk of bias by standardising the pre- study meal, carefully considering how the test meal was presented to the participants and ensuring that levels of activity were similar between the two arms of the study.

One limitation of this study was that the participants' weight was not measured again before and after each week of intervention and therefore it was not possible to comment on changes in body weight, if any. However given the minimal difference in energy intake and comparable step count (a proxy for energy expenditure) between the two arms of the study, a change in weight would not have been expected. Adding other anthropometric measures such as body circumferences and skinfold thickness would have been interesting. As in the previous chapter, the collection of blood and analysis for hormone assays was beyond the scope and funding available for this study but would be a useful addition to future work to aid in the interpretation of the glucose and appetite data. In this study the participants were free living during the 7 day intervention which has an ecological validity but conversely could introduce variability in the responses.

5.4.2 Conclusions:

The Addition of LAGG to the cooking process of white jasmine rice decreased postprandial blood glucose levels compared to plain white rice in a healthy adult population, in keeping with previous study. The beneficial effect of GG was sustained and a significantly reduced peak blood glucose was notes at 1 week in the order of 1.2 mmol/L. Establishing that any acute effect noted is sustained over a period of consumption will be important prior to recommending the addition of LAGG to rice cooking as a therapeutic or public health strategy.

6 Discussion

The work carried out successfully proved the underlying hypotheses: jasmine rice meals cooked with LAGG reduced rice starch digestion *in vitro*, reduced glucose responses *in vivo* in an acute setting, and that this effect was sustained after 1 week of daily exposure. The effects on appetite and gastrointestinal responses were more nuanced.

This work set out to investigate whether a rice cooking process using hydrocolloids could modify the physiological responses to a white rice meal. The idea originated from limited evidence in the literature. There are some reports linking the addition of hydrocolloids to rice-based materials to *in vitro* starch digestibility. This has been shown for example for hydrocolloids pullulan, xanthan gum and guar gum (210, 211). Also, it has been shown in an *in vivo* mice model that locust bean gum and agar reduced starch digestibility (212). A report from Japan (137) showed that cooking white 'Koshiibuki' rice in xanthan gum, a hydrocolloid of bacterial origin, reduced the glycaemic response to a rice meal compared with rice cooked on its own in healthy adult female students. This appears to be the only work demonstrating that modification of rice processing by adding a hydrocolloid of bacterial origin flattens the blood glucose response in humans. Hydrocolloids of bacterial origin are a category of food hydrocolloids which is still under-researched. In the systematic review and narrative synthesis carried out by the investigator, the evidence linking their use, either as direct food supplements or

incorporated into various foods, to modulate a reduction in blood glucose was mixed (213) and warranted further investigation.

The specific choice of food hydrocolloid LAGG for this work was based on previous experience gained by collaborators in Birmingham and Copenhagen. This preliminary work (148) suggested that LAGG may reduce rice starch digestibility *in vitro* and also obstruct disintegration of simulated rice boluses. The latter observation is in keeping with the MRI appearance of the meal in the stomach as described in Section 3.3.5, which demonstrates the powerful synergy of using bench models and *in vivo* imaging methods and comparing the findings. In addition to this, LAGG appeared a particularly suitable candidate for this work as it was a hydrocolloid of bacterial origin such as the xanthan gum of the report mentioned above (137), and it is colourless and tasteless. Also, the acidic environment of the stomach may strengthen the LAGG gel, making it stronger. The specific choice of jasmine white rice for this work was made based again on preliminary experience from our collaborators and widespread consumption. The results of the *in vitro* study indicated that the GI of jasmine white rice was 94. This finding was consistent with literature GI values reporting a GI range from 96 to 116 for different jasmine rice, values that are classified as a high GI food (173). The exact mechanism as to why LAGG lowers postprandial blood glucose values is not entirely clear, but there are several possible explanations. The first is that during cooking the gum coats the surface of the rice with a LAGG layer which as a result makes the starch less accessible to the amylase enzymes in the digestive system. This coating of the rice was discovered

by our collaborators in Birmingham and Copenhagen. Novel use of a 5-DTAF-labelled gel resulted in clear visualisation of the LAGG in the fluorescent microscopy images on the outer surface and penetrating into the grain rice after cooking. This protective layer could result in the starch being broken down into glucose more gradually which consequently decreases the glucose spike postprandially and overall decreases the iAUC. This is also supported by research that reported that guar galactomannan, which is also another common polysaccharide like gellan gum, may form a layer around starch granules which then makes it harder for enzymes to digest them (214). The second possible explanation is that the gum may also affect directly the enzyme amylase by binding to it during the process of digestion and this, as a result, may slow glucose digestion down further. As mentioned in Chapter 1, amylase has an essential role in the breakdown of starch, it is found in the saliva where it initiates the breakdown and later in the pancreatic secretions, which continue the breakdown into glucose in the small bowel, ready for absorption. This is supported by a study that found guar galactomannan had a direct non-competitive inhibitory effect on alpha-amylase (215). In this study, it was suggested that the binding of the enzyme to galactomannan resulted in the galactomannan-amylase complex being inactive. This could potentially apply to GG and amylase due to the composition of guar and GG being similar, but it remains to be demonstrated. Therefore, although this work has confirmed the main hypothesis that the addition of LAGG flattens the postprandial blood glucose response, the exact mechanism requires further research.

In this work in the acute intervention study the change in GI did not modulate significantly GE. It has been shown that carbohydrates with different digestion rates incrementally decreased GE rates in an animal models, mediated by the ileal break mechanism (216).

The impact on appetite, whilst interpreted with great caution as differences were not significant, raises some interesting questions. In Chapter 4, the acute feeding study, numerically LAGG appears to result in less hunger and more fullness post- prandially. However, in Chapter 5, numerically the reverse is seen postprandially in response to the acute test, and numerically the consumption of rice with LAGG for 7 days appears to amplify this. In contrast, over the intervention period numerically there is lower hunger at the start and end of the day with LAGG than with the control rice. These points require further investigation with larger samples sizes. Should LAGG slow digestion, then this might impact on the available nutrients to stimulate appetite suppressing gut hormones produced by nutrients in the small intestine, immediately after consumption, but on the gradually degradation of the LAGG gel the nutrients might become available reflected in the apparent sustained satiety.

The SPMIC's new, open design Paramed 0.5T MRI scanner could have allowed imaging of the stomach in the upright, more physiological position. However, it was felt that the current limited imaging spatial resolution and contrast that the upright scanner can provide were not ideal for our interest in exploring possible rice bolus retention in the stomach. Conversely, the conventional, horizontal bore 1.5T MRI

scanner provided resolution and image contrast suitable for our needs, as shown in Figure 4.14.

The ingestion of NSP gum have been reported to have an effect on colonic fermentation which may result in the production of SCFA (217). For example, a study that examined the effect of different food hydrocolloids in mice reported that pectin and guar gum increased SCFA levels (218). SCFA have been reported to have an important role in the stimulation of the hormonal and neural response which affect in turn appetite and energy intake. A study reported that SCFA acetate has a direct effect on appetite regulation (219). Animal studies suggest that SCFA stimulate the release of hormones PYY and GLP-1 from L cells from colon and leptin from adipose tissue and contribute to reduced appetite (220).

In regards to GG, there is one *in vitro* fermentation study which evaluated the role of GG on colonic fermentation and reported that GG influenced the gut microbiota and promoted the production of SCFA (221). To date, no human studies are available. Breath hydrogen reflects carbohydrate fermentation in the colon. Carbohydrates that are not absorbed in the small intestine enter the colon and are fermented by bacteria in the colon resulting in the production SCFA and release of gases such as carbon dioxide hydrogen and methane (222). The breath hydrogen test has been used to assess the colonic fermentation (223). Therefore, the use of breath hydrogen test to evaluate the effect of GG on colonic fermentation in this present work could have provided a valuable tool and contributed to the understanding of fermentation mechanisms.

Non-alcoholic fatty liver disease (NAFLD) is a major consequences of a high fat diet. In a mice experiment, GG reduced hepatic triglyceride and body fat mass which has been induced by a high fat diet (221). Furthermore, a high GI diet was shown to increase hepatic fat and glycogen stores in eight healthy individuals (104). A review of four studies involving 281 patients diagnosed with NAFLD showed that low GI diet significantly reduced hepatic fat mass and alanine transaminase hepatic enzyme (224). Therefore, the addition of GG to high GI foods could provide a health benefit with respect to both glycaemic response and lipid profile .

One particular strength of this work was the logical development from *in vitro* characterization and choice of the intervention to *in vivo* acute dose, to sustained intervention. Randomised controlled feeding studies testing an intervention over a number of days compared with baseline are not very common in the field albeit they are necessary to evaluate sustained efficacy of interventions (204).

The impact of this work could be wide-ranging. The modification of the cooking process is safe, simple, and cheap and could potentially provide an intervention to help slow down the rising levels of obesity and T2DM that have been linked to increasing consumption of white rice. Whilst it remains to be demonstrated, one could predict that this methodology could apply to a variety of starchy foods to alter the glycaemic and gastrointestinal responses. Whilst grains have been studied, the impact of incorporating LAGG in foods in which the grains have been milled, hence exposing starch granules would be of interest. Improved

knowledge in this area may help to direct evidence-based dietary advice to benefit patients and the general population.

6.1 Future research and directions based on these findings

This work can develop in several future directions. Future work could focus on those living with pre- diabetes, diabetes and obesity, whose responses to the modified rice meals are unknown. It would be interesting to strengthen the evidence of possible positive health effects after a sustained period of consumption. This would be essential prior to recommending the addition of LAGG to rice cooking as a therapeutic or public health strategy.

The addition of LAGG to other foods would be of interest, for example, where it is incorporated into the structure such as a baked product with more potential to coat the starch grains, or whilst cooking, such as pasta or potatoes. Different rice varieties and other grains including ancient grains, like millet (225), could be explored. Other areas for future development could include considering texture analysis of the rice grains cooked with and without LAGG, meal volume effect, ethnicity of the participants, and also more complex studies whereby the whole diet of the participants is provided and controlled.

Based on these data future studies could be powered with an appropriate sample size to investigate appetite and gastrointestinal effects of the rice meal with LAGG. Blood glucose responses could be monitored using an interstitial continuous glucose sensor. Studies taking postprandial blood

samples for full hormonal assays could help dissect the glucose metabolic physiology responses to the rice + LAGG intervention compared with the rice control meal. Other improvements to the work presented here could include anthropometric measurements of weight, waist circumference and skin fold thickness before and after sustained interventions. Use of electronic food record data collection would streamline data analysis.

6.2 Conclusions

Modifying the cooking process of jasmine white rice with LAGG reduced the GI of the rice in an *in vitro* model of digestion and reduced blood glucose responses in healthy humans both after an acute and a sustained intervention. These data are novel and add to knowledge in the field. The modification of the cooking process is safe, simple, and cheap and could potentially provide an intervention to help reduce the post prandial glucose response to white rice, potentially impacting on the rising levels of obesity and T2DM seen in populations consuming white rice as a staple food.

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8 Appendices

8.1 Instructions for food diaries

INSTRUCTIONS FOR FILLING IN YOUR FOOD DIARY

- Please keep a food diary for the rest of the study day only. The record should include as much detail as possible.

- Fill in the food diary each time you consume a drink, a meal or a snack. Please note the time, and describe everything you eat or drink. Use a further page if you need more space.

- When you record a food or drink, please give a thorough description and include any Brand names. You cannot give us too much detail! For example:-
    - Was the bread white/wholemeal/granary
    - Did you have a baguette/cob/pitta/slice?
    - Did you use butter or margarine, was the margarine made with olive/sunflower oil?
    - Was the product a diet product- e.g. sugar-free lemonade?
    - Was anything added- e.g. sugar to tea, butter to toast, tomato ketchup or mayonnaise etc?
    - Do you use full fat/semi-skimmed/skimmed milk?
    - How did you cook your food – eg. boiled/fried/baked/raw?
    - How was the food processed – eg. fresh/tinned/frozen?
    - Was the product Heinz/Tesco's own etc.?

- The amounts you have should be described as fully as possible using household measures
    - Use kitchen equipment – a measuring jug, a mug (of know volume), a teaspoon (see below) and a tablespoon

a level teaspoon

a rounded teaspoon

a heaped teaspoon

    - Look on cans and packets to see whether the weight/volume is given and describe how much of the packet you had (eg. Half a 400g can of Heinz tomato soup)
    - Record the number of sausages/fish fingers/bacon rashers etc. that you ate
    - Describe the size of a piece of food – eg. egg sized potato, small matchbox sized piece of cheese

- Please record each item of food separately. If you drink white tea with sugar, please record the type and amount of tea, milk and sugar separately.

- If you had a packed lunch, each part must be recorded in the diary.

- It is important that you complete the 'leftovers' section with anything you did not actually eat. Please give us as much detail as possible

- For homemade recipes, the raw ingredients should be recorded in the back of the diary in the 'Recipes' section. Please tell us what the meal is composed of and how many it served. You can then put an entry in the diary such as "one quarter of the spaghetti Bolognese made with recipe 1".

8.2 Food diaries

Example				
Time	**Type of food or drink with brand names and cooking method**	**Amount**	**Food or drink left over and amount**	**Office use only: code and weight**
7.30am	Beans on toast:			
	Heinz baked beans (reduced sugar and salt)	3 heaped tablespoons		
	White toast (Warburtons)	2 thick slices		
	Flora spread	2 heaped teaspoons		
	Cup of tea:			
	PG tips tea	300ml		
	Semi-skimmed milk	50ml		
	Sugar	1 teaspoon		
10.30am	Dairy Milk chocolate	40g bar		
	Diet coke	330ml can		
12.45pm	Ham sandwich:			
	Ham (Tesco Honey Roast)	3 thin slices	¼ of a slice	
	White bread (Warburtons)	2 thick slices		
	Flora spread	2 heaped teaspoons		
	Tomato	½ beef tomato		
	Cup of tea (as above)	300ml		
	Walkers Salt & Vinegar crisps	30g bag		
	Granny Smith apple	medium		
7.15pm	1/4 of spaghetti Bolognese made using recipe 1			
	John Smiths beer	2 pints		
	Mr Kipling Bramley Apple pie	2 individual pies		
9.00pm	Hot chocolate made with semi- skim milk	2 teaspoons chocolate/ ½ Pint of milk		

Subject ID -……..... Date…….. Day:

Time	Type of food or drink with brand names and cooking method	Amount	Food or drink left over and amount	Office use only: code and weight

Subject ID..... Date ..... Day:

Recipe no: 1	**Dates used:**	Serves: Please also indicate on food diary the proportion of recipe you had on each occaision	**Name of recipe:**
Ingredient (with brand names and cooking method)		**Amount**	**Comments**

8.3 Written consent form for acute intervention study

Healthy Volunteer's Written Consent Form 1

Full Study Title: Effects of gellan gum on the glycaemic, gastrointestinal and appetitive responses to a white rice meal assessed by MRI

Please initial the box for each statement

- I voluntarily agree to take part in this study. ☐
- I confirm that I have been given a full explanation by the above named and that I have read and understand the information sheet given to me which is attached. ☐
- I have been given the opportunity to ask questions and discuss the study with one of the above investigators or their deputies on all aspects of the study and have understood the advice and information given as a result. ☐
- I agree to comply with the reasonable instructions of the supervising investigator and will notify him immediately of any unexpected unusual symptoms or deterioration of health. ☐
- I authorise the investigators to disclose the results of my participation in the study but not my name for example to the audiences of scientific presentations and publications. ☐
- I understand that information about me recorded during the study will be kept in a secure database. If data is transferred to others it will be made anonymous. Data will be kept for at least 10 years after the end of the study. ☐
- I authorize the investigators to disclose to me any abnormal test results. ☐
- I understand that the image of my body may be used in future studies (approved by an Ethics Committee) beyond the scope of the study explained here. Any images used will be made anonymous, and I will not be identifiable. ☐
- I understand that I can ask for further instructions or explanations at any time. ☐
- I understand that I am free to withdraw from the study at any time, without having to give a reason for withdrawing. ☐
- I confirm that I have disclosed relevant medical information before the study. ☐

Optional: Consent for storage and possible use in future research

- I agree that the data gathered about me can be stored by the rice research team at the University of Nottingham for possible use in future studies. I understand that some of these studies may be carried out by researchers other than current team of Miss Norah Alshammari, Dr Luca Marciani (PI), Who ran the first study, including researchers working for commercial companies Any samples, scans or data used will be anonymized, and I will not be identified in any way. ☐

- I shall receive an inconvenience allowance of £40 for each visit, totalling £80 for the 2 visits, plus a bonus of £20 on completion including returning the food diaries as planned (grand total on completion £100). If I withdraw from the study for medical reasons not associated with the study I will receive an inconvenience allowance proportional to the length of the period of participation, but if I withdraw for any other reason, the inconvenience allowance to be received, if any, shall be at the discretion of the investigator. ☐

- I have not been a subject in any other research study in the last three months which involved: taking a drug; being paid a disturbance allowance; having an invasive procedure (eg venepuncture >50ml, endoscopy) or exposure to ionising radiation. ☐

Abnormal Findings in Scans

- I understand that the SPMIC is not a clinical diagnostic facility and so does not routinely inspect images for abnormalities. I understand that my MR scans will NOT routinely be reviewed by a radiologist (or any other medically qualified person) to look for any signs of disease, and it is unlikely that any abnormalities that may be present will be detected. ☐

- On the other hand I understand that if one of the investigators should happen to notice something on my scan which they think is abnormal then they will show my scans to a medically qualified doctor who will contact me if further action is required. ☐

- I understand that if an abnormality were detected on my scan it may affect my ability to get life insurance. ☐

- I understand that if the study investigators feels it necessary to inform my GP of my participation in the study, or of an adverse event or abnormal finding on my scan, I understand I am giving my consent to do so ☐

GP Name: _____ Telephone number: _____ Surgery Address _____

Full Name: ……Telephone: ……
Address……Signature:……Date……

To be filled in by an Investigator
I confirm that I have fully explained the purpose of the study and what is involved to:
……
I have given the above named a copy of this form together with the information sheet.

Investigators Signature ……Date……Name …… Ethics Code ……
Volunteer Number.

Please return one copy of this form to the SPMIC and retain one copy in the study master file

8.4 General Health checklist

INCLUSION CRITERIA (Subjects must answer YES to the following questions in order to qualify for the study)		
	Yes	**No**
Does the subject have no medical conditions which might affect study measurements (judged by the investigators)?	☐	☐
Is the subject aged ≥ 18; ≤ 65?	☐	☐
Does the subject have a body mass index (BMI) ≥ 18.5 and ≤ 24.9 kg/m$^{2?}$	☐	☐
Is the subject able to give informed consent?	☐	☐
EXCLUSION CRITERIA (Subjects must answer NO to the following questions in order to be deemed eligible for the study)		
Does the subject have an eating disorder as indicated by the Three factor eating questionnaire and SCOFF?	☐	☐
Has the subject participated in another nutritional or biomedical trial 3 months before the pre-study examination or during the study?	☐	☐
Is the subject not used to eating breakfast?	☐	☐
Is the subject not used to eating three meals a day?	☐	☐
Has the subject reported participation in night shift work during the two weeks prior to pre-study investigation or during the study? Night work is defined as working between midnight and 6.00 AM.	☐	☐
Does the subject do strenuous exercise for more than 10 hours per week?	☐	☐
Does the subject consume ≥21 alcoholic drinks in a typical week?	☐	☐
Has the subject reported weight loss or gain ≥ 10 % of bodyweight during the six months period before the pre-study examination?	☐	☐
Is the subject following a medically- or self-prescribed	☐	☐

diet during the two weeks prior to the pre-study examination and until the end of the study?		
Does the subject dislike the products served as the dietary test treatments?	☐	☐
Has the subject got any allergy or food intolerance to the test treatments?	☐	☐
Does the subject use medication which interferes with study measurements (as judged by the study investigator)?	☐	☐
Is the subject not suitable for MRI scanning (e.g., presence of metal implants, infusion pumps and pacemakers) as assessed by standard MRI safety questionnaire?	☐	☐
(For females) Does the subject declare to be pregnant?	☐	☐
Has the subject had antibiotic or prescribed probiotic treatment in the past 12 weeks?	☐	☐
Does the subject have Inability to lie flat?	☐	☐
Does the subject exceed scanner limits of weight <120kg?	☐	☐
Does the subject understand English language?	☐	☐
Name (please print):……. Signed: …….. Date (dd/mm.yyyy):…….		

8.5 Screening SCOFF questionnaire

Please tick the yes/no answer that applies.

	YES	NO
1. Do you make yourself sick because you feel uncomfortably full?		
2. Do you worry you have lost control over how much you eat?		
3. Have you recently lost more than one stone (14 pounds) in a 3 month period?		
4. Do you believe yourself to be fat when others say you are too thin?		
5. Would you say that food dominates your life?		

Study ID:…….

Staff Signature _____ Date _____

8.6 MRI safety screening questionnaire

Name:	Scan Date:	Date of Birth:
Address:	Volunteer Number:	
	Ethics Code:	
Phone number:	Weight:	Height:

MR scanning uses strong magnetic fields. For your own safety and the safety of others it is **very important** that you do not go into the magnet halls with any metal in or on your body or clothing. Please answer the following questions carefully and ask if anything is not clear. All information is held in the strictest confidence.

Do you have any implants in your body *(e.g. replacement joints, drug pumps)*? ***Please provide details overleaf***	Y/N
Do you have aneurysm clips (clips put around blood vessels during surgery)?	Y/N
Do you have a pacemaker or artificial heart valve? *(These stop working near MR scanners)*	Y/N
Have you ever had any surgery? ***Please give brief details overleaf*** *(We do not need to know about uncomplicated caesarean delivery, vasectomy or termination of pregnancy)*	Y/N
Do you have any foreign bodies in your body *(e.g. shrapnel)*?	Y/N
Have you ever worked in a machine tool shop without eye protection?	Y/N
Do you wear a hearing aid or cochlear implant?	Y/N
Could you be pregnant? *(Pregnancy tests are available in the female toilets)*	Y/N
Have you ever suffered from tinnitus?	Y/N
Do you wear dentures, a dental plate or a brace?	Y/N
Are you susceptible to claustrophobia?	Y/N
Do you suffer from blackouts, epilepsy or fits?	Y/N
Do you have any tattoos? ***If yes, you may be asked to read and sign another form***	Y/N
Do you have any body piercing jewellery that cannot be removed?	Y/N
Do you have any skin patches (trans-dermal patches)?	Y/N
Do you have a coil in place (IUD) for contraception? Do you know what type?	Y/N
Do you have any condition that may affect your ability to control your temperature? *(e.g. Do you have a fever, cardiovascular disease, hypertension, diabetes or cerebrovascular disease?)*	Y/N
Will you remove all metal including coins, body-piercing jewellery, false teeth hearing aids etc before entering the magnet hall? (*Lockers are available by the changing rooms)*	Y/N
Is there anything else you think we should know?	Y/N

8.7 Study day eligibility check questionnaire

For this project we wish to study people who are well today and who have followed the study instructions and restrictions we discussed at enrolment. To ensure this we ask you to complete the following questionnaire. Please circle your answer All your answers will be held in the strictest confidence.

Have you taken alcohol in the last 24 hours?	Yes	/	No
Have you taken caffeine (tea, coffee, cola) in the last 18 hours?	Yes	/	No
Have you performed any strenuous exercise in the last 24 hours?	Yes	/	No
Have you eaten or drunk since last night at 10pm (apart from a glass of water before 7 am today)?	Yes	/	No
Have you taken any dietary supplement or medication of any kind in the last 24 h (including aspirin, paracetamol, codeine, vitamin pills, etc)?	Yes	/	No
If yes please specify			
Are you well at the moment?	Yes	/	No

8.8 Satiety quotients for the acute intervention study

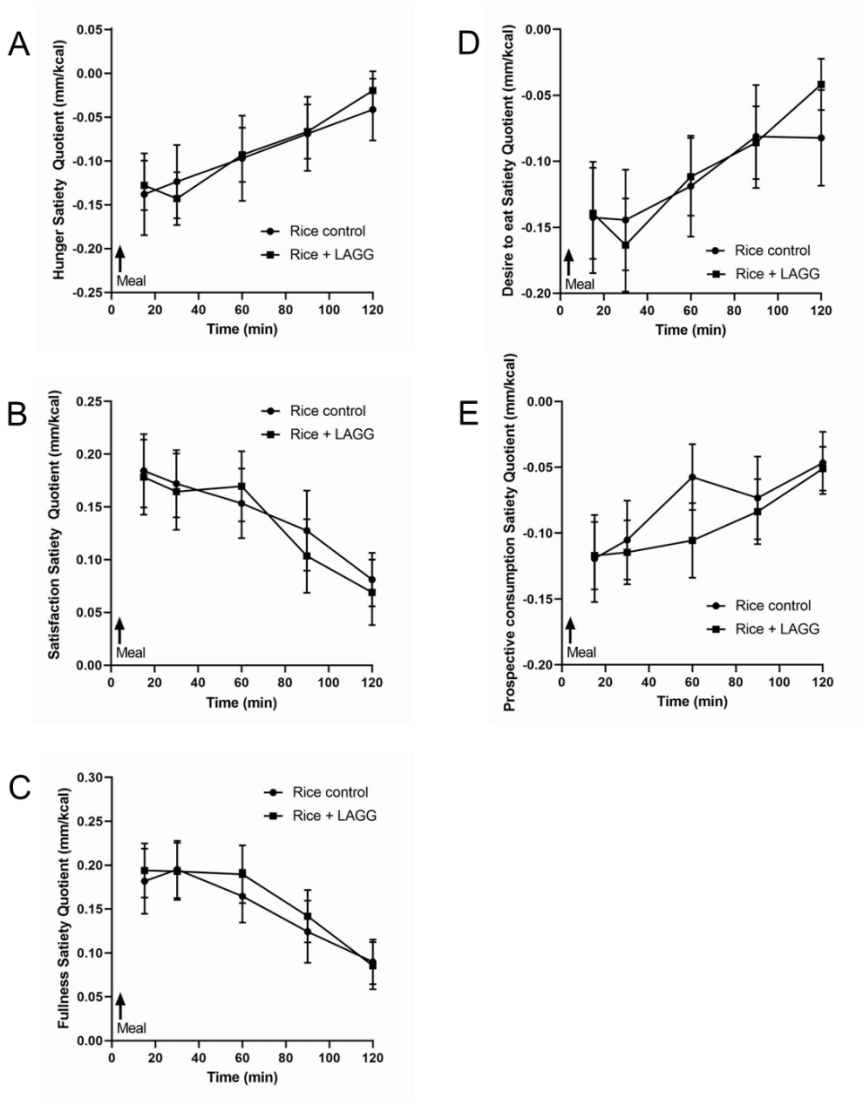

Figure 8.1 Satiety Quotient time courses from N=12 healthy participants who consumed the rice meal with and without the addition of low acyl gellan gum (LAGG) during the cooking process. The panel shows the SQs calculated for each visual analogue scale collected during the MRI study day: A) hunger, B) satisfaction, C) fullness, D) desire to eat and E) prospective consumption. Data points are mean ± SEM.

8.9 Consent form for sustained intervention study

CONSENT FORM
(Final version 1.0: 28/11/2021)

Title of Study: **The Effects of gellan gum on the acute glycaemic and appetitive responses to a white rice meal and impact on energy intake over a 7 day period.** REC ref:(**414-1121**)

Name of Participant: **Please initial box**

1. I confirm that I have read and understand the information sheet version number ……dated…… for the above study and have had the opportunity to ask questions. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason. I understand that should I withdraw then the information collected so far cannot be erased and that this information may still be used in the project analysis. ☐
3. I understand that relevant sections of data collected in the study may be looked at by authorised individuals from the University of Nottingham, the research group and regulatory authorities where it is relevant to my taking part in this study. I give permission for these individuals to have access to these records and to collect, store, analyse and publish information obtained from my participation in this study. ☐
4. I understand that information about me recorded during the study will be made anonymous before it is stored. It will be uploaded into a secure database on a computer kept in a secure place. Data will be kept for 7 years after the study has ended and then destroyed. ☐
5. **Optional**: I agree that the research data collected about me may be stored and used in possible future research during and after 7 years, and shared with other researchers including those working outside the University. ☐
6. I understand that the tests or procedures are carried out for research only and not for clinical diagnostic purposes. However, if the study investigator should feel it necessary to inform my GP of my participation in the study, or of an adverse event or abnormal test result, I understand I am giving my consent to do so. ☐
7. I have not been a subject in any other research study in the last three months which involved: taking a drug; being paid a disturbance allowance; having an invasive procedure (eg blood sample >50ml) or exposure to ionising radiation. ☐
8. I voluntarily agree to take part in the above study. ☐

Name of Participant	Date	Signature
Name of Person taking consent (if different from Principal Investigator)	Date	Signature
Name of Principal Investigator	Date	Signature

2 copies: 1 for participant, 1 for the project notes.

8.10 Restrictions to diet and lifestyle on the day before each sustained intervention study day

Dietary restrictions the day before your study day:

- Please avoid caffeine (tea, coffee, cola) for 18 hours prior to your study day.
- Please avoid alcohol for 24 hours prior to your study day.
- Please avoid taking any dietary supplement (e.g. multivitamins and minerals pills, iron supplements) for 24 hours prior to your study day.
- Please eat the meal that we have given you on the night before your study day and then please start fasting (i.e. have no further food or drink other than water) from 9pm that night. A glass of water is allowed on waking up on the study day but not after 7am. Please do not eat or drink anything else until your reach the study centre for 9 am (i.e. you will have fasted for 12 hours and had nothing to drink after 7am)

Lifestyle Restrictions the day before scanning:

- Please avoid performing any strenuous exercise for 24 hours prior to your study day. This includes avoiding jogging or cycling briskly to the study centre on the day of your study.

8.11 Satiety quotients for the sustained intervention study

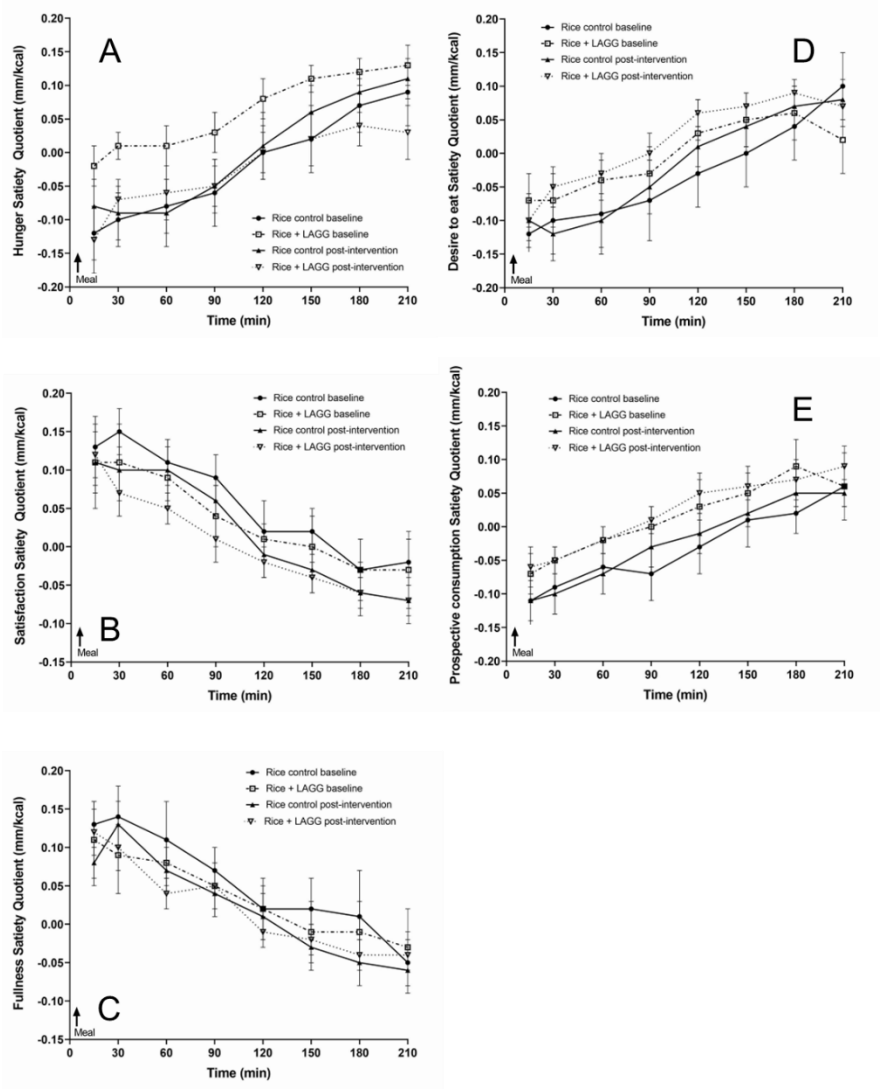

Figure 8.2 Satiety Quotient time courses from N=8 healthy participants who consumed the rice meal with and without the addition of low acyl gellan gum (LAGG) during the cooking process. The time courses are shown both for the study day at baseline and for the study day after 1 week of repeated intervention (termed post-intervention).

The panel shows the SQs calculated for each visual analogue scale collected during the MRI study day: A) hunger, B) satisfaction, C) fullness, D) desire to eat and E) prospective consumption. Data points are mean ± SEM.