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The role of seaweed bioactives in the control of digestion: Implications for obesity treatments

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Seaweeds are an underutilised nutritional resource that could not only complement the current western diet but potentially bring additional health benefits over and above their nutritional value. There are four groups of seaweed algae; green algae (Chlorophyceae), red algae (Rhodophyceae), blue-green algae (Cyanophyceae) and brown algae (Phyophyceae). Seaweeds are rich in bioactive components including polysaccharides and polyphenols. Polysaccharides content, such as fucoidan, laminarin, as well as alginate is generally high in brown seaweeds which are also a source of polyphenols such as phenolic acids, flavonoids, phlorotannin, stilbenes and lignans. These components have been shown to reduce the activity of digestive enzymes, modulating enzymes such as α-amylase, α-glucosidase, pepsin and lipase. This review discusses the effect of several of these components on the digestive processes within the gastrointestinal tract; focusing on the effect of alginate on pancreatic lipase activity and its potential health benefits. Concluding that there is evidence to suggest alginate has the potential to be used as an obesity treatment, however, further in vivo research is required and an effective delivery method for alginate must be designed.
Introduction

There are four groups of seaweed algae; green algae (Chlorophyceae), red algae (Rhodophycae), blue-green algae (Cyanophyceae) and brown algae (Phyophyceae). Seaweeds as a whole have been suggested as a source of “antiviral, antibiotic, anti-thrombic, anti-coagulant, anti-inflammatory, anti-lipaemic, anti-cancer and enzyme-inhibiting agents” which have been reviewed elsewhere. Brown seaweeds are rich in polysaccharides such as fucoidan, laminarin, as well as alginate. Laminarin has shown bioactive properties in the GI tract, inducing changes in mucin sulphation/sialation. Fucoidans are found in brown seaweed and invertebrates, with fucoidans from invertebrates having a simple ordered structures as compared to the complex structures found in seaweed. In humans, fucoidan from Fucus vesiculosus inhibits sperm-egg binding by affecting sperm binding to the glycoprotein membrane (zona pellucida) of the oocyte. Fucoidans have also been found to inhibit Helicobacter pylori adhesion to gastric mucosa, reduce lipid accumulation in adipocytes in vitro and show antioxidant and anti-inflammatory properties. The structure of fucoidans is far from being fully understood, and so the relationship between structure and function of bioactive fucoidan is also not fully understood.

Alginate is a polysaccharide indigestible to humans and as such can be considered a dietary fibre. Found in cell walls and intercellular space of brown seaweed (Phaeophyceae), alginate can also be produced by some bacteria of the Azotobacter and Pseudomonas genii as a component of the extracellular matrix. Work in our lab and elsewhere has shown alginates can reduce the activity of the digestive enzymes pepsin and pancreatic lipase in vitro.

Bioactive factors such as polyphenols (phenolic acids, flavonoids, stilbenes and lignans) from both red and brown seaweeds have demonstrated α-amylase and α-glucosidase inhibition. The major polyphenol found in seaweed is phlorotannin. Phlorotanin is composed of up to 8 phloroglucinol monomers and three types are found in the Fucacaea family of seaweeds; fucols, fucophlorethols and phlorethols.

Bioactive Alginate

Alginates are unbranched polysaccharides composed of (1→4)-α-L-guluronic acid (G-residues) and (1→4)-β-D-mannuronic acid residues (M-residues). In seaweeds these polyuronans are found as salts of different metals (usually sodium and calcium). The polyuronic chains are composed of blocks, of which are either G rich, M rich, or mixed (Figure 1). The characteristics of the alginate are dictated by the arrangement of these blocks. G-rich blocks are relatively stiff as there is limited rotation around the glycosidic bond. The presence of mannuronic acid residues increases chain flexibility with M blocks and MG structures forming relatively flexible chains because of freer rotation around the glycosidic bonds.

There are two mechanisms for alginate gel formation, either interchain binding of divalent cations forming ionic gels or through lowering the pH below the pK_a of the alginate can cause acid-gel formation.

In the food industry, alginates are used as thickening, gelling, foaming, emulsifying and stabilisation agents. Alginates also have medical and scientific applications; cell and drug encapsulation, controlled delivery systems, adsorbent wound dressings as well as an anti-reflux therapy. Oligo-G alginates have also been shown to have anti-bacterial properties, disrupting biofilm structure and growth. Oligo-G alginates have also been shown to affect the mucus gel and are being investigated as a potential therapy helping Cystic Fibrosis sufferers to clear mucus from their airways.

Lipases

Human pancreatic lipase is a 46 kDa enzyme produced in the exocrine pancreas and secreted along with bile from the liver. The active site of the pancreatic lipase is composed of a catalytic serine-histidine-aspartate triad which is well conserved throughout the lipase family.
The α-helices and the β-strands are arranged in an orientation common to all lipases, termed the α/β hydrolase fold. All lipases are single domain enzymes, with the exception of pancreatic lipase, which needs a co-protein (colipase) for activity in the presence of bile salts or detergents. Work in this lab has shown that pancreatic lipase maintains considerable activity in vitro even in the absence of colipase; however it is unable to function without the presence of bile salts.\(^{27}\)

There are two conformations for the lipase; either as the open, active conformation; or as the closed, inactive form. The closed conformation is due to a loop or ‘lid’ that covers the entrance to the active site serine. In the case of human pancreatic lipase there are two moving loops, one large (termed the lid comprising of 24 amino acids), one small (9 amino acids) and a stabilising third loop that does not move (10 amino acids).\(^{26}\) The moving loops both have to undergo a conformational shift to allow entry of the substrate into the active site.

Colipase, an 11,000 Da protein, reverses the inhibitory effect of bile salts and detergents at the water-lipid interface. Lipase has only been imaged in the open conformation when colipase is bound.\(^{28}\) It is known that colipase is not the activating factor, as in the absence of bile salts and detergents colipase is not required for activity. However, in physiological conditions when lipase is at the water-lipid interface the open lid does make multiple contacts with the colipase.\(^{28}\)

Lipase is believed to penetrate into the micelle or droplet and sequester lipid for hydrolysis. The lid and colipase form a hydrophobic area sufficient for penetration.\(^{28}\) The lipid is likely to enter the active site in a ‘tuning fork’ orientation\(^{27}\) (Figure 2), with one acyl chain (one prong) in the active site and the second acyl chain (second prong) running along the outside of the lipase molecule in a groove created by two phenylalanine residues.\(^{28}\)

The presence of a calcium binding site is classed as one of the specific structural features of a pancreatic lipase, however, no absolute requirement for calcium has been shown for pancreatic lipase.\(^{30}\) Contrary to this, Zangenberg et al (2001), state that calcium is necessary for the activity of pancreatic lipase and the rate is highly dependent on the concentration.\(^{31}\) Yet within the same study the group clearly showed lipase activity in the absence of calcium. Alternatively since both the calcium binding sites are well removed from the active site, the role of calcium may be purely structural.\(^{32}\) However, Yang et al (2000) show that the stability of the enzyme is independent of calcium.\(^{32}\)

A second possible method for the increased rate of hydrolysis in the presence of calcium ions may be due to the formation of Ca\(^{2+}\) soaps with the fatty acids, resulting in a precipitate.\(^{33}\) The precipitate may remove the potentially inhibitory effect of free fatty acids on triacylglycerol (TAG) hydrolysis.\(^{31}\) In vitro, a crystalline envelope composed of Ca\(^{2+}\) soaps can form around the micelle or oil droplet; however intensive stirring removes the envelope.\(^{31}\) It is likely that similar stirring like forces would be present in the GI tract.

Another potential role for calcium ions would be to reduce the electrostatic repulsion between the enzyme and the interface.\(^{33}\) Wickham et al. (1998) showed that the addition of calcium ions did reduce the surface charge of the emulsion droplets in the presence of bile salts.\(^{34}\) The evidence appears to suggest that the role of calcium (if it is essential) is of structural importance and not one that directly affects the catalytic site.

**Fat Digestion**

The major source of dietary fat is TAG which makes up 90-95% of dietary fat.\(^{35}\) Remaining fat sources comprise a mixture of phospholipids, glycolipids and sterols.\(^{35, 36}\) Fat digestion is initiated in the mouth; mastication begins the mechanical dispersion of fats and the formation of food in to a bolus. Lingual lipase is secreted from a set of lingual serous glands on the tongue called von Ebner’s glands, in response to a meal.\(^{37}\) Chewing serves to mix lingual lipase in with food bolus which is passed into the stomach through swallowing.\(^{36, 38}\) Lingual lipase has a pH optimum of 5.5 but is resistant to acid inactivation.\(^{39}\) Lipase activity is therefore retained in the stomach when the pH environment is buffered with the intake of a meal.\(^{38, 40}\)
Gastric lipase is secreted into the stomach from gastric peptic cells. It is believed that 10-30% of dietary fat is digested in the stomach before passage into the small intestine. The stomach is also responsible for creating a crude emulsion of dietary fats, through churning and initial lipolysis which then pass into the duodenum.

The first step of TAG digestion is the hydrolysis to diacylglycerol (DAG). Gastric and Lingual Lipase both preferentially cleave the fatty acid at the SN3 position, Figure 2). The fatty acid at SN1 is cleaved sequentially, leaving an SN2-Monoacylglycerol (SN2-MAG). The spontaneous rearrangement of the SN2-fatty acid to position SN1 can allow for the complete hydrolysis into glycerol and free fatty acids.

As lipase acts at the lipid-water interface, the level of emulsification is an important factor in the rate of fat digestion as it determines the area over which lipase can act. The breakdown products of lipids including fatty acids, cholesterol and phospholipids bile acids form mixed micelles. As the mixed micelles pass through the small intestine pancreatic lipase acts to further digest dietary fats.

Three types of obesity have been described: (i) metabolic obesity; where identifiable syndromes or diseases result in weight gain, (ii) socio-cultural obesity; where historically obesity may have been seen as a status symbol or sign of wealth and (iii) Environmental obesity; which encompasses the modern epidemic where otherwise physiologically normal individuals become obese.

Managing obesity through exercise and diet is the preferred treatment due to lower cost and risk of complications. However, the long term efficacy of dieting as a treatment has been questioned, in a review of dietary studies, Ayyad et al. (2000), suggest an average long term success rate of just 15% for dietary treatment.

Bariatric surgery has proved to be the most successful intervention. Gastric bands, gastric bypass, gastric reduction surgery and intra gastric balloons all seek to physically reduce the capacity of the stomach. A meta-analysis of 136 studies accounting for 22,000 patients showed that significant weight loss was achieved in 61% of all types of bariatric surgery. A comorbid improvement of diabetes, hyperlipidaemia, hypertension, and sleep apnoea was also observed. However, in the UK, bariatric surgery is normally only considered for those with a BMI greater than 40, or for patients with a BMI between 35 and 40 and a comorbid condition which would benefit.

A number of anti-obesity agents have been suggested as medical treatments of obesity. However, due to side effects, many of these agents are not approved for use, for example, phenylpropanolamine, fenfluramine, methamphetamine, and amphetamine. Orlistat, a pancreatic lipase inhibitor, is the most commonly prescribed obesity medication in the UK. A randomised double-blind study showed that when used in conjunction with a calorie restricted diet, orlistat can cause a mean weight loss of 5.9% of body mass compared with 2.3% for those on a calorie restricted diet and placebo. However, side effects including steatorrhea and faecal incontinence, can make it an unpleasant treatment for the patient.

Orlistat (Figure 3) is a semi synthetic hydrogenated derivative of natural occurring compound from Streptomyces toxytricini, which has been shown to inhibit gastric and pancreatic lipase.

Orlistat binds to the active site of pancreatic lipase, resulting in irreversible acylation of a hydroxyl group on serine residue. In human studies enzyme inhibition greater than 90% has been reported, without affecting trypsin, amylase, chymotrypsin and phospholipases, even though trypsin and chymotrypsin have a serine at the active size of the enzyme.

Modulation of digestion by dietary fibres

Hemicellulose, pectin and xyl have been shown to inhibit trypsin (up to 80% inhibition) with pectin and cellulose inhibiting α-amylase up to 35%, and pectin and cellulose inhibiting pepsin by up to 60%.
Rats fed a high fibre diet containing 20% cellulose have shown a significant decrease in intestinal proteolytic, lipolytic and amylolytic enzyme activity\(^{57}\). Dilution of stomach contents with dietary fibre has been suggested as a possible factor during in vivo studies of enzyme activity\(^{57}\). However, the same investigators were also able to demonstrate in vitro inhibition of pancreatic enzymes in samples of human pancreatic juice. With the exception of pectin, the fibres examined (alfalfa fibre, oat bran, hemicellulose, wheat bran and cellulose) all brought about a reduction in enzyme activity, with cellulose and hemicellulose producing the largest effect\(^{58}\).

El Kossiri et al (2000), measured casein digestion with pancreatin in the presence of a range of soluble fibres including carrageenan, locust bean gum, alginate and pectin. The dietary fibres brought about a reduction of protein digestion which was shown not to be related to viscosity.\(^{59}\)

Work from our laboratory has demonstrated that dietary fibres possess the ability to alter digestion in the gastrointestinal tract. Sunderland et al (2000) demonstrated in vitro pepsin activity could be inhibited by alginate by 52%.\(^{60}\) This could be increased to 89% inhibition, dependent on the structure of the alginate. A negative correlation was seen between pepsin inhibition and G residue but a positive correlation with alternating blocks of G and M,\(^{11}\) possibly due to the increased flexibility between the bond of alternating M and G residues.\(^{61}\)

**Alginate Inhibition of Lipase**

Further work within this laboratory has showed that specific alginites were capable of inhibiting pancreatic lipase up to 72.2% (± 4.1) using a synthetic substrate DGGR (1,2-di-lauryl-rac-glycero-3-glutaric acid-(6’-methylresorufin) ester) and 58.0% (± 9.7) with a natural substrate (olive oil TAG).\(^{12}\)

The inhibitory effect was shown to be related to alginate structure, with alginites high in guluronic acid shown to be more potent inhibitors of pancreatic lipase. High-G alginites extracted from the *Laminaria hyperborea* seaweed inhibited pancreatic lipase to a significantly higher extent than high-M alginites from the *Lessonia nigrescens* species (Figure 4). The alginate technology as an inhibitor of pancreatic lipase is now under patent, and is being investigated as an anti-obesity agent in human trials.\(^{52}\)

Alginate showed potent inhibition of fat digestion in both of the assays (using synthetic and natural substrates), however it is possible that the inhibition of pancreatic lipase is substrate specific, and favours the inhibition of particular TAG and that there may be a relationship between fatty acid chain length and degree of inhibition. The way in which alginate interacts with TAG of different fatty acid chain lengths is being investigated elsewhere.

Alginate is not the only biopolymer that has been shown to inhibit the activity of pancreatic lipase. Wilcox (2010) also showed that certain pectins, were also capable of inhibiting lipase in vitro.\(^{63}\) Pectins were capable of inhibiting lipase activity by up to 24.7±6.3%, and this was shown to be related to levels of esterification.\(^{63}\) Kumar et al (2010) argue that the carboxyl groups of pectin interact with the active site residues of the lipase enzyme, protonating them and disrupting the catalytic mechanism.\(^{64}\) This explains why increasing levels of esterification reduce inhibition, as the number of free carboxyl groups is decreased. If this is true, then a similar mechanism for alginate inhibition of lipase maybe possible as they are similarly rich in carboxyl groups.

Molecular weight of alginate was not a determining factor of lipase inhibition (Figure 5) and neither was viscosity as one of the best inhibitors (F[G]= 0.633, MW=34,700), had a viscosity of 6 mPas compared to a poor inhibitor (F[G]=0.424, MW=221000), which had a viscosity of 121 mPas (for 1% solution in phosphate buffered saline). However it appeared that a minimum molecular weight was needed to inhibit lipase. Recent research from this laboratory has shown that low molecular weight fractions (below 5,000 Da) of M or G blocks or a mixture of the two had little effect on lipase activity when assessed using the methodologies of Wilcox et al (2014) [data not shown].\(^{12}\) Briefly, the methodology used 1,2-di-lauryl-rac-glycero-3-glutaric acid-(6’-methylresorufin) ester (DGGR) as the substrate for lipase and the activity was assessed as an increase in the absorbance over time, when measured at 575nm.
Several potential mechanisms for this inhibitory effect have been suggested. Alginate has the potential to interact with both the substrate and the enzyme itself. Alginate is a negatively charged polymer, capable of forming electrostatic interactions with positively charged proteins at low pH.\(^\text{65}\) Alginate may associate with protein through hydrogen bonding at hydroxyl groups; charge-charge interactions with \(\delta\)-carboxyl groups, and the negatively charged COO- group of the alginate, although this group would become protonated at low pH. The pH sensitivity of the synergism between alginate and proteins suggests that these electrostatic interactions are important in inhibition. Alginites with a high G block content are known to interact with glycoprotein, specifically mucin measured by rheological assessment across a range of mucin: alginate ratios.\(^\text{66}\) It was hypothesised that alginate can interact with specific sites along the protein section of the glycoproteins, cross linking several mucin molecules together forming a gel.\(^\text{66}\)

The role of calcium on the activity of pancreatic lipase is unclear, and because alginate can sequester calcium, the authors have carried out further investigations. From structural information there appears to be a calcium ion binding site involving four residues in a nine residues loop (Glu188 to Asp196) along with two water molecule.\(^\text{30}\) There is a second calcium molecule buried in the Cys181 region of lipase and held in place by five water molecules.\(^\text{30}\) Alginate can chelate divalent cations and therefore may remove potentially important calcium molecules from the enzyme.

When using the lipase activity assay, as described by Wilcox et al (2014), the activity of lipase, in the absence of added calcium, was 80.4% (±3.7) of the activity in the standard assay (8.6 µM), this difference was not significant. Figure 6 showed that increasing the calcium concentration (up to 171.3 µM) had no effect on lipase activity, using the same test with differing calcium additions. However, above 171.3 µM Ca\(^{2+}\) the activity of lipase does drop off with increasing concentrations of calcium, to a minimum of 68.5% (±1.1), showing that the highest calcium concentrations can significantly reduce the activity of the enzyme.

If alginate was inhibiting lipase by binding calcium, it would be expected that the inhibition would be overcome by the addition of further calcium. However the levels of lipase inhibition by alginate at low concentrations of calcium (0-171.3 µM) are not changed greatly. Maximum inhibition of 54.7% (±12.7) was seen at the standard concentration of calcium (8.6 µM). The lowest level of inhibition (42.6% (±1.5)) was seen at 171.3 µM. Even when the concentration of calcium was increased to 685.7µM, alginate was still capable of inducing 39.8±4.8% inhibition (Figure 7).

Lipase inhibition by alginate is unlikely to be due to calcium binding by the biopolymer as inhibition remains constant (40% or greater) through the calcium range.

### Alginate as a weight management tool

Alginites have previously been shown to increase fatty acid excretion in ileostomy patients, in a small study of six ileostomy subjects. This was believed to be a result of entrapment with the alginate matrix.\(^\text{67}\) The increase in fatty acid excretion may now be explained by the alginites capacity to inhibit lipase and therefore reduce the amount absorbed by the body. Alginites have been used in the food and pharmaceutical industry for many years for functions other than enzyme inhibition. The inclusion of an alginate into foods (without altering taste or acceptability) may have the potential to reduce the uptake of dietary TAG and could greatly help in weight management.

Data from previous research suggests that alginate, as a dietary fibre, may be used as an obesity treatment, however the main obstacle appears to be how to introduce alginate into the everyday diet. The addition of alginate to food vehicles is not a new concept and has been developed since the early 90s with the addition of alginate to food and drink resulting in a reduction in glycaemic response,\(^\text{68}\) a reduction in blood glucose, reduced gastric emptying,\(^\text{69}\) increased fat excretion,\(^\text{67}\) and a reduction in Kcal intake.\(^\text{70}\) Despite these beneficial effects, alginate enriched products are not always of high palatability. Ellis et al (1981) reported that foodstuffs that contain viscous fibres usually exhibit slimy, sticky and gummy characteristics resulting in poor palatability and therefore poor compliance.\(^\text{71}\)

An alginate white bread has been developed within our laboratory; including alginate up to 4% wet weight of dough. The bread produced was of a high standard, which was not noticeably different to a
standard white loaf. Alginate was shown to be released from the bread matrix at the initial stages of digestion in the small intestine, where the majority of TAG digestion occurs.\textsuperscript{72} The baking process used in the manufacture of the bread has also been shown to affect the molecular weight of the alginate but does not alter the inhibitory properties.\textsuperscript{73}

Further beneficial effects

Alginate have also been shown to have specific health benefits. The effects of alginate and other dietary fibres on GI health are summarised in Table 1.

Inhibition of Lipase by Seaweed Extracts

The bioactive components have been shown to inhibit digestive enzymes but it has also been shown that whole seaweeds can have a similar effect. The benefit of including whole seaweeds rather than the extracted bioactives would be the reduction in the need for processing, the increase in fibre content, as well as other bioactives and the inclusion of seaweed minerals, such as iodine. However, taste and acceptability would still need to be overcome for the seaweed based products to become widely accepted.

In collaboration with workers from the Cardiovascular, Diabetes and Nutrition Research Centre in Kuala Lumpur, work in this lab showed that extracts of three species of tropical red algae from Malaysia (\textit{Kappaphycus alvarezii}, \textit{Kappaphycus striatus} and \textit{Eucheuma denticulatum}) are capable of inhibiting lipase activity \textit{in vitro}.\textsuperscript{14} Figure 8 showed that the ethanol extracts of all of the dried seaweed brought about a significant reduction in lipase activity, with 83-92\% inhibition.\textsuperscript{14}

Figure 8 also shows that the ethanol extraction process is not essential to inhibition, with the dried seaweed powder of all three seaweed species; \textit{Kappaphycus alvarezii}, \textit{Eucheuma denticulatum} and \textit{Kappaphycus striatus}, significantly inhibiting lipase activity by 61, 60 and 67\% respectively. Red algae are a rich source of polyphenols and natural antioxidants and it has previously been shown that phenolic compounds can inhibit digestive enzyme activity, including that of lipase. The ethanol extract of \textit{Eucheuma denticulatum} also significantly inhibited α-amylase activity by 88\%.

Soluble fibre extracts of all three seaweeds brought about reductions in lipase activity, with the soluble fibre extracts of \textit{Kappaphycus alvarezii}, and \textit{Eucheuma denticulatum} bringing about significant reductions in lipase activity of 60\% and 57\% respectively as shown in Figure 9.\textsuperscript{14}

Conclusion

There is a sizeable body of research reporting that dietary fibre can affect digestion, and may possess enzyme inhibitory properties. This evidence along with the beneficial nutritional and health related benefits associated with dietary fibre suggests that alginate may be able to be used in the treatment of obesity and aid in weight loss, without the undesirable side effects associated with current pharmacological obesity treatments.

Dried seaweed and ethanol extracts also show lipase inhibition, but dried seaweed added to foods is likely to have palatability problems and ethanol extract rich in polyphenols but poor in fibre could well produce the same side effects as orlistat.

Although there is compelling evidence to suggest alginate does have the potential to be used as an obesity treatment, further \textit{in vivo} research is required, and an effective delivery method for alginate must be designed.
References


Figure 1  Structure of alginate. Upper is the chain conformation and the lower are the two sugar residues that make up the alginate structure β-D-mannuronic acid and α-L-guluronic acid. Figure adapted from Draget et al (2002). 20
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Figure 9 Effects of soluble fibre (V) extracted from (dried seaweed) *K. alvarezii* (Ka), *E. denticulatum* (Ed) and *K. striatus* (Ks) at the concentration of 3.8 mg mL$^{-1}$ on pancreatic lipase activity. Commercially available alginate (CAA) at 3.8 mg mL$^{-1}$ was included as comparison. Lipase enzyme as a control was set at 100 %, and all the other values were normalised to this lipase enzyme control value, respectively. Orlistat was used as a positive reference. The data represent mean ±SEM of three independent assays (n=3). Asterisk denotes $P<0.05$ compared with the control. One-way ANOVA is followed by Bonferroni’s test for post hoc analysis. Figure modified from Balasubramaniam *et al.*$^{14}$
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Table 1  The Gastrointestinal effects of consumption of alginate.
Potential Health Benefits of Whole Seaweed

Antiviral, antibiotic, anti-thrombic, anti-coagulant, anti-inflammatory, anti-lipaemic, anti-cancer, enzyme inhibition

Potential Health Benefits of Alginate

Lipase inhibition, pepsin inhibition, reduced fat digestion, reduced glycaemic response, delayed gastric emptying, reduced plasma cholesterol, improved GI health