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The role of food structure in gastric-emptying rate, absorption and metabolism

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The high levels of non-communicable diseases such as CVD and type 2 diabetes mellitus are linked to obesity and poor diet. This continuing emphasis on health in relation to food is proving a powerful driver for the development of cheap but palatable and more functional foods. However, the efficacy of such foods is often hard to prove in human subjects. Thus, a suite of tools has been developed including *in silico* and *in vitro* simulations and animal models. Although animal models offer physiologically relevant platforms for research, their use for experimentation is problematic for consumers. Thus, *in vitro* methods such as Infogest protocols have been developed to provide digestion endpoints or even an indication of the kinetics of digestion. These protocols have been validated for a range of food systems but they still miss the final absorption step. This review discusses the use of such *in vitro* models and what further steps need to be included to make the bioaccessibility determination more relevant to bioavailability and human health.

Key words: *In vitro* digestion: Functional foods: Non-communicable disease: Digestion kinetics

There is increasing evidence that the food we eat needs to be healthier and more sustainable. Henry Dimbleby in his National Food Strategy (https://www.nationalfoodstrategy.org/) highlighted the issue. The same range of drivers is also pushing the trend for more minimally processed food, while concern about additives and a lack of understanding of food ingredients is leading to classification of some foods as 'ultra processed'[1]. At the same time, climate concerns associated with animal production are driving trends in lower consumption of animal-based foods: meat, dairy, eggs, etc. in favour of more plant-based foods. Despite these concerns about nutritional quality and sustainability, the food production system must continue to feed everyone all of the time. These concerns may have arisen because as a population, we do not value our food sufficiently or in a way that balances nutrition and sustainability with cost, safety and palatability, which are the primary drivers of consumer choice. For much of the population, food choice is driven by palatability and cost so consequently these have become the main drivers for retailers and fast-food outlets[2]. It has been suggested that in addition to providing dietary advice, the food suppliers should be making more of health by stealth approaches[3]. There are already a number of examples where the food industry has been able to make significant changes to formulations of staple products such as the reduction of salt in bakery products[4]. There is similar work ongoing to

Abbreviations: GI, glycaemic index; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide 1.

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increase very gradually the amount of fibre in a range of food products. Although there may be evidence correlating a low-fibre intake with disease, we can take a more objective measure such as glycaemic index (GI) as a measure of dietary quality rather than fibre content. Not least because the current definitions of dietary fibre are unhelpful[5]. In a recent article, Jenkins *et al.* showed that the risk of CVD among the study participants increased with dietary GI. This was accentuated in those with a BMI over 25 kg/m^2[6]. Specifically, for those with a BMI less than 25 kg/m^2 the hazard ratio for the top GI quintile was 1·14(SD 0·14), while for those with a BMI over 25 kg/m^2 the hazard ratio for the top quintile was 1·38(SD 0·16). It is noteworthy that the average BMI of middle-aged (55–64) UK adults is 28·1 kg/m^2[7]. Similarly, there is a strong correlation between dietary GI and risk of type 2 diabetes mellitus. Local dose dependence of the relative risk of type 2 diabetes mellitus on the GI in prospective cohort studies combined showed that the dose–response type 2 diabetes mellitus–GI risk relation rose by 32 % per 0·1 increment in the GI[8].

Although we have evidence that dietary GI correlates with non-communicable diseases, the mechanism is less clear. What we know is that the GI is linked to digestion kinetics[9]. The main site of control of digestion kinetics is the gastric compartment because the stomach acts as a container for the food that is consumed in a meal and releases it in a controlled way into the small intestine, which is the primary site of digestion and absorption. The rate of gastric emptying is governed by a number of factors, specifically the textural properties of the gastric chyme and the nutrients being delivered to the duodenum[10]. Thus, liquids empty faster than more solid meals and low-energy foods empty faster than high-energy foods. In this context water has a short gastric residence time while a nutrient-dense solid meal will have a long residence time. In addition, there is a link between the blood glucose concentrations and gastric emptying, with higher concentrations decreasing gastric-emptying rates[11]. The control of gastric emptying is done through a number of mechanisms. Glucose absorption in the small intestine induces a feedback loop via cholecystokinin, peptide YY, glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) which are secreted from the intestine in response to nutrient exposure. GLP-1 and GIP induce the release of insulin and GLP-1 inhibits glucagon secretion, which attenuates postprandial glycaemic excursions. At the same time, the blood glucose concentration modulates gastric emptying, such that acute elevations of blood glucose levels slow gastric emptying (effects are evident even within the physiological range) and emptying is accelerated during hypoglycaemia.

Food structure and gastric emptying

As an illustration of some of these effects, we undertook a study to determine the extent to which oat particle size in a porridge could alter glucose absorption, gastric emptying, gastrointestinal hormone response and subjective feelings of appetite and satiety[12]. In a crossover design, eight participants were fed porridge prepared from either oat flakes or oat flour with the same protein, fat, carbohydrate and mass. Subjective appetite ratings, gastric contents and plasma glucose, insulin and gastrointestinal hormones were determined over a period of 3 h post-consumption. The use of MRI provides direct visualisation of gastric content: changes in gastric emptying and also what is emptied. As an example, Fig. 1A and 1B shows MRI images for 5 and 25 min, respectively, post-consumption of oat flake porridge. The abdominal cross-sections show that most of the 175 ml liquid consumed with the 264 g porridge was emptied within 25 min of consumption. This highlights that structure is important in defining what is emptied. Regardless of the early emptying of gastric liquid from the oat flake porridge, its structure meant that after 3 h post-consumption the oat flake porridge had an average 25 % greater volume remaining in the stomach than the starch porridge. Despite the limited differences in the rate of gastric emptying, significant differences were seen in plasma GIP and insulin and minor differences in GLP-1. The peak in GIP and GLP-1 was at 20 min post-consumption for the starch porridge and 35 min for the flake porridge. The peak timing was reversed for insulin and no differences were seen in plasma glucose. Thus, highlighting that in healthy individuals blood glucose concentrations are tightly constrained even when very different amounts are being absorbed as suggested by the differences in GIP.

Similarly, structural effects can be seen in the digestion and absorption of protein and lipids. Indeed food structures can be tailored to alter the timing of the delivery of specific macronutrients. Making use of density differences to drive creaming or sedimentation of components can be particularly effective[13]. When participants consumed a liquid or semi-solid meal with the same fat, protein, carbohydrate and energy as shown in Fig. 1C and 1D, differences in emptying behaviour were seen. The dark shapes in the stomach in Fig. 1C are boluses of high fat and protein cheese formed in the mouth and the swallowed. These remained visible for up to an hour before dispersing, trapping the protein and fat at the bottom of the stomach. In contrast, the liquid meal already showed evidence of creaming of the fat to the top of the stomach after 5 min. The food boluses were measured and compared with particle-size distributions from other meals, which highlighted the influence of the different meal structural properties on gastric chyme[14]. Thus in the first hour the composition of the gastric chyme emptied into the small intestine would have been very different. This was confirmed rather circumstantially as the gastric-emptying rate for the liquid meal at 35 min post-consumption was significantly faster. The differences in emptying then led to differences in gastric volume and subjective appetite scores after 3 h. In another study using MRI to compare the effects of energy density and viscosity on gastric-emptying rate[15], the authors found that increasing the viscosity

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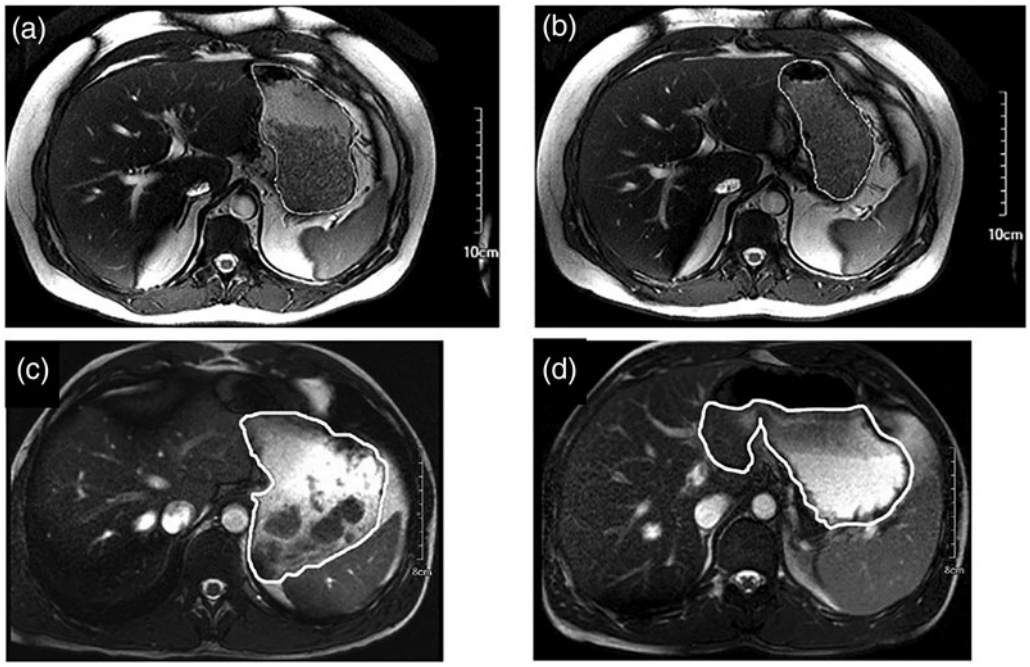

Fig. 1. Axial FIESTA (Fast Imaging Employing Steady-state Acquisition) MRI images of the stomach (outlined) taken 5 min (A) and 25 min (B) post-consumption of oat flake porridge. Image (A) shows a layer above the oat flake porridge that is not apparent after 25 min (B). Axial TrueFISP (True Fast Imaging with steady state precession) MRI images of the stomach (outlined) taken 5 min after consumption of a semi-solid (C) or liquid (D) version of the same meal.

was less effective at slowing gastric emptying than increasing the energy density. However, the viscosity was more important in increasing the perception of fullness. The results highlighted the lack of satiation from 'empty energy' in quickly ingested drinks such as fizzy drinks. Although these examples highlight the role of food structure in digestion kinetics, it is also apparent that measuring the bioactive concentrations in peripheral blood cannot tell the whole story because we do not know what was being absorbed. In order to fill this gap, a number of simulations of digestion have been developed to mimic human physiology and enable the digestive fate of bioactives to be more closely followed.

In vitro simulations of the influence of food structure on digestive fate

The Infogest network has been a key player in developing physiologically relevant and widely usable *in vitro* simulations of food digestion. The network was originally assembled through a COST Action (FA1005) that had protein digestion as a core consideration in relation to food allergy[16]. However, this was soon broadened to include all food and macronutrient types. The most widely cited simulation protocol is the static model that uses fixed conditions to simulate upper gastrointestinal tract digestion[17,18]. This simulation has been used in hundreds of studies and comprises three phases, oral, gastric and intestinal. It can provide valuable information on digestion endpoints for any bioactives where microbial fermentation does not play a role. The oral phase includes simulated salivary fluid, salivary amylase and recommendations for how the chewing of food should be simulated. The oral phase generates the food bolus that is passed into the stomach for further digestion. The gastric phase of the simulation includes simulated gastric fluid as well as the protease pepsin and a recommendation for the use of gastric lipase. The recommendation is for this phase to last for 2 h at pH 3, which is an estimate of mean pH values achieved *in vivo* when half of a meal has been emptied from the stomach into the duodenum. Although this is the recommendation, there is some evidence that the pH should be higher, perhaps closer to 5[19] depending on the nature of the meal. This is important because it determines which gastric enzymes are active. Salivary amylase is active in the stomach at higher pH, gastric lipase in the mid-range of pH and the protease pepsin at low pH. After 2 h, chyme from the gastric phase is emptied into the intestinal phase for digestion to be completed. The intestinal simulation includes simulated intestinal fluid, bile and pancreatic enzymes incubated at pH 7 for 2 h. This kind of approach allows a range of different endpoints to be determined, depending on the nutrients or bioactives of interest. These might include determination of peptide profiles or free amine groups for protein digestion, maltose concentrations for starch and NEFA profiles for lipid digestion. It should be highlighted that this is a static model using fixed parameters and thus is unlikely to give physiologically relevant kinetic data. However, it can be used for assessing digestibility of nutrients such as protein[20] and is a strong alternative to replace animal models for determining protein

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nutritional quality. The key requirements of this simulation of the upper gastrointestinal tract are reproducibility and physiological relevance. The reproducibility has been confirmed in a number of ring trials[21] and the physiological relevance has been demonstrated in a number of different systems[22–24]. However, it should be noted that this type of simulation is only able to model luminal events and it lacks brush border enzymes and absorptive elements.

As highlighted earlier, it has become apparent that it is not just the extent of digestion that is important for disease but also the rate of digestion and nutrient release. Thus, it is important to have ways of determining digestion kinetics and this can be achieved using simple semi-dynamic simulations of digestion[25] or more sophisticated computer-controlled simulations[26]. The most important phase of digestion to simulate in relation to digestion kinetics is the gastric phase. Thus, kinetic simulations tend to concentrate on this phase and control factors such as gastric loading, gastric emptying, acidification rate, enzyme and simulated gastric fluid secretion rate and physical processing of the gastric chyme. The examples shown in this article draw on the Infogest semi-dynamic protocol that has been used to determine the impact of food structure on digestion kinetics but there are many other similar models in the literature.

In a study undertaken in 2012 and published in 2013, the effect of the structure of dairy products was initially investigated[13]. In this study, we were able show that the semi-solid structure persisted in the stomach and suppressed gastric emptying over the first hour compared to the liquid meal. This difference then led to persistent differences in volume of gastric contents and associated feelings of fullness for up to 3 h post-ingestion. Although this study hints at the role of food structure in digestion kinetics in support of previous work[27], it does not directly show that the rate of appearance of the products of digestion varied. In order to determine that kind of information, studies must either use intubation[28] or resort to animal or *in vitro* models. In a subsequent study the Infogest semi-dynamic model was used to follow the digestion of the same two meals in more detail[29]. In those simulations, the detailed analysis of protein and lipid digestion was able to show that gastric behaviour was affected by the initial structure with creaming and sedimentation observed in the case of liquid and semi-solid samples, respectively. Lipid and protein digestion profiles showed clear differences in the amount of nutrients reaching the simulated small intestine and, consequently, the likely bioaccessibility after digestion. The semi-solid sample generated higher nutrient released into the small intestine at an early stage of digestion whereas nutrient accessibility from liquid sample was delayed due to the formation of a cream layer in the gastric phase. This shows the strong effect of the matrix on gastric behaviour, proteolysis and lipolysis.

In two similar studies, dairy processing (heating and/or homogenisation) was used to alter the microstructure of cows' milk prior to simulated digestion[30,31]. Both studies showed the typical clotting behaviour of whole milk in the gastric phase of digestion. They also showed differences in clot consistency depending on the processing applied to the milk. Unprocessed 'raw' milk had the firmest clot while homogenised and heated and homogenised presented weaker clots. In particular, the study by Mulet-Cabero *et al.* showed that the clot from raw or heated milk was dense enough to sediment, while the homogenised and heated and homogenised curd entrapped sufficient lipid to cream to the top of the gastric compartment. These structural changes occurring during the gastric phase resulted in different nutrient emptying, with significant differences between 'raw' and heated and homogenised, and more extensive digestion of milk proteins in the heat-treated samples due to the drastic denaturation of the proteins.

This research has shown that *in vitro* digestion can provide a platform for linking food structure to digestion[32,33]. The use of *in vitro* simulations of digestion driven by human study data provides a powerful tool to improve the nutritional quality of food. Most recently, both MRI and *in vitro* simulation have been combined to non-invasively follow the influence of food structure on digestion[34–36]. In all cases, it is the combination of a range of approaches linking human study data to physiologically relevant *in vitro* studies that has broadened understanding of the role of food structure in digestion kinetics.

Validation of simulations of digestion

It is clearly important for any model to be validated against data from the system that it is replicating. In the case of *in vitro* models of human digestion, this can often be problematic[37]. The rationale for using *in vitro* simulations of digestion is that they can provide direct information about the breakdown of food in the gastrointestinal tract that is often hard to gather in human studies. This can in turn improve interpretation of results from nutritional epidemiological studies that necessarily generate correlative outcomes. There are numerous examples of the use of *in vitro* digestion to determine the fate of specific macronutrients but much of the research focuses on protein and starch. The move to more plant-based diets has led to research on protein quality using the FAO-recommended digestible indispensable amino acid score system[38,39]. The Infogest simulation of digestion had also been used to provide the source data for digestible indispensable amino acid score[20], with the benefit no animals are involved. However, the *in vitro* results were validated against results from experiments *in vivo.* The validation was made by comparison of seven different protein sources with data collected both in pigs and in human subjects[40]. The results showed good agreement between the results *in vivo* and the Infogest *in vitro* results but as the authors note, the comparison was only made with a limited set of food sources, so no general conclusions about the efficacy of the Infogest approach can be made for assessing all protein quality. The digestion of protein has been investigated in relation to food allergy, in particular because it has been suggested that stability

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to digestion may be a key parameter for a protein to be an allergen[41]. However, the outcomes are highly dependent on the nature of the *in vitro* digestion model being used and its relevance to specific allergens[42,43]. In particular, the European Food Safety Authority currently uses a late phase gastric model with low pH and high protease activity but this is very different from an infant simulation[44,45]. As a result, there have been calls for European Food Safety Authority to review the methodology for novel protein risk assessment[46]. The examples given here are for the Infogest-recommended simulations but there are many others in the literature with increasing levels of sophistication and focusing on different bioactives[47,48].

In addition to protein, there has been a lot of interest in understanding the role of processing and structure of starch in digestion relating to glycaemic response[49,50]. Although comparison can be made with *in vivo* data, as highlighted by Phillips *et al.* earlier[11], there are many individual factors that can make such a comparison problematic, not least the fact that glucose in peripheral blood is not a good indicator of bioaccessible glucose in the gut[51]. Because starch is the most important digestible polysaccharide in human nutrition usually accounting for 20–50 % of the total energy intake, it has been studied extensively[52]. The apparent health benefits of a low-GI diet led the Carbohydrate Quality Consortium to state 'an urgent need to communicate information on GI and GL'[53]. Consequently, it is important to note that a number of studies have shown the validity of the *in vitro* determination of the GI[50,54,55]. The ability of resistant starch to pass through the upper gastrointestinal tract and into the colon has also highlighted the need for more sophisticated models of human digestion that include colonic fermentation.

What more is needed?

As the pressure to reduce animal experiments increases there a drive to find suitable models to replace them[56,57]. As a result, there has been a proliferation of *in vitro* models of digestion, many of which are more sophisticated in the way that they mimic the physical and biochemical environment of the gut[58]. These include simulations of the gastric phase[59,60] where the biochemical and physical environment of the stomach are replicated, or the gastric and intestinal phases[61] or the gastric, intestinal and colonic phases of digestion[62]. However, these simulations lack the final stages of digestion and any absorption steps. Although some attempts have been made to include brush border enzymes[63], these have not been widely accepted because difficulties in defining activity and exposure time.

There are many *in vitro* models focusing on just the colonic phase of digestion often based on the early work of MacFarlane *et al.*[64]. These are becoming increasingly important in enabling research to understand the role of dietary fibre and plant bioactives in relation to gut microbiota and the gut–brain axis[65]. However, the issue with investigating the role of colonic fermentation in the digestion of complex foods using *in vitro* approaches is the carry-over of compounds from the small intestinal phase to the large intestinal fermentation phase. Transporters in the apical membrane of enterocytes often specifically control absorption in the small intestine. Thus, both highly nutritive molecules such as simple sugars, amino acids and peptides as well as fatty acids are largely removed from intestinal chyme. Additionally, potentially toxic bile acids are reabsorbed in the distal ileum. These and similar absorption mechanisms are not simulated well by passive dialysis so improvements need to be found in presenting realistic digesta to simulations of colonic fermentation.

In silico models of digestion are also becoming more widely available[66,67] but many of them are focused only on protein digestion using specific rules for modelling the action of proteases. However, more generally applicable *in silico* models of digestion will only become reliable when more human study data are made available to refine them[68]. With the rise of machine-learning approaches, this is likely to become a more tractable approach in the future.

Conclusions

A number of non-communicable diseases have digestion kinetics as underlying risk factors. Thus, disease is undoubtedly related to dietary quality suggesting that consumers need to build a better relationship with their food. The evidence presented here shows that food structure can affect gastric-emptying rate and digestion kinetics. This confirms that food structure as well as composition is important in risk factors for non-communicable diseases. The link between food and digestion kinetics can be studied in more detail using *in vitro* simulations validated using human data. Such models can help provide preliminary data on the slower digesting, more functional foods that are needed to decrease the prevalence of non-communicable diseases.

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Conflict of Interest

None.

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Authorship

A. Mackie is the sole author of this article and no authors who would reasonably be considered an author have been excluded.

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