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Symposium two: Novel methods for assessing protein metabolism

Monitoring food digestion with magnetic resonance techniques

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This review outlines the current use of magnetic resonance (MR) techniques to study digestion and highlights their potential for providing markers of digestive processes such as texture changes and nutrient breakdown. *In vivo* digestion research can be challenging due to practical constraints and biological complexity. Therefore, digestion is primarily studied using *in vitro* models. These would benefit from further *in vivo* validation. NMR is widely used to characterise food systems. MRI is a related technique that can be used to study both *in vitro* model systems and *in vivo* gastro-intestinal processes. MRI allows visualisation and quantification of gastric processes such as gastric emptying and coagulation. Both MRI and NMR scan sequences can be configured to be sensitive to different aspects of gastric or intestinal contents. For example, magnetisation transfer and chemical exchange saturation transfer can detect proton (^{1}H) exchange between water and proteins. MRI techniques have the potential to provide molecular-level and quantitative information on *in vivo* gastric (protein) digestion. This requires careful validation in order to understand what these MR markers of digestion mean in a specific digestion context. Combined with other measures they can be used to validate and inform *in vitro* digestion models. This may bridge the gap between *in vitro* and *in vivo* digestion research and can aid the optimisation of food properties for different applications in health and disease.

Digestion: Gastric emptying: MRI: Protein

The process of digestion is necessary for acquiring nutrients from the foods we ingest. Digestion encompasses a series of complex physiological, mechanical and biochemical processing steps that lead to the mechanical and biochemical breakdown of food structures which ultimately allows absorption and utilisation of nutrients[1]. Both independent and interrelated processes at multiple length scales are involved in food breakdown, mixing and absorption[2]. Briefly, anticipation of food intake triggers several anticipatory physiological responses such as increased salivation and production of gastric juice that prepare the body for the influx of nutrients[3]. The first phase of digestion is the oral phase (ingestion). During oral processing, mastication

Abbreviations: CEST, chemical exchange saturation transfer; GE, gastric emptying; MR, magnetic resonance; MT, magnetisation transfer; RF, radiofrequency.

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and secretion of saliva lead to the formation of a food bolus that can be swallowed safely[4]. These oral processes are not only important for digestion, but also for sensory perception of foods, eating enjoyment and satiation[5]. The second phase is gastric digestion, during which the food mass in the stomach is prepared for further digestion and absorption in the intestines. Gastric digestion involves mixing and addition of hydrochloric acid along with pepsin (a protease) and gastric lipase[6]. The third phase is intestinal digestion; from the stomach food passes through the pyloric valve into the small intestine where pancreatic proteases, lipases and amylase are added along with bile and the resulting chyme is mixed. The chyme is passed along the small intestine where most of the nutrients are absorbed. The small intestine comprises the duodenum, jejunum and ileum and chyme proceeds from one to the other until it passes through the ileocaecal valve into the large intestine. There, some of the remaining undigested food such as dietary fibre is fermented by bacteria into absorbable compounds such as SCFA, and most of the remaining water is removed before defecation[1]. Across and within these different processing stages there are numerous physiological signals (neural and hormonal) that feed forward and backward, presumably to optimise digestion.

In vivo digestion research can be challenging due to practical constraints, biological complexity and ethical obstacles. Notably, classic techniques to study digestion *in vivo* are mostly invasive and involve for example taking gastric aspirates through a nasogastric tube or monitoring gastric pressure or pH with sensors. Therefore, *in vitro* model systems are widely used to study digestive processes under controlled and simplified conditions[7]. This provides detailed information on the effects of enzymatic processes on the physical and chemical characteristics of food structures during digestion[8,9]. Although validation and refinement of *in vitro* models using *in vivo* data is an ongoing collaborative effort in the field (see https://www.cost-infogest.eu/ and Bohn *et al.*[10]), bridging the significant gap between *in vitro* model systems and the complexity of *in vivo* digestion remains a challenge. An emerging approach that could help address this in part involves the use of ingestible devices that can take samples or measurements as they pass through the gastrointestinal tract[11,12]. The core idea that will be explored in the present paper is that magnetic resonance (MR) techniques may be used to bridge this gap because they can be used to monitor relevant digestive processes both *in vitro* and *in vivo*.

NMR provides information on the state of water protons in foods and has been widely used as a characterisation and process quality control tool in different food systems[13,14]. It can be performed at relatively low magnetic field strengths (about 0·5 T) and is used for measuring *in vitro* samples of digesta or gastric aspirates. It has the advantage of low cost and ease of operation. MRI is a commonly used related technique that, among numerous other applications e.g. in medicine, can be used to perform both *in vitro* and *in vivo* imaging measurements[13] non-invasively. It is most commonly performed at 1·5 or 3 T and is more expensive than NMR. NMR and MRI share the same underlying principles and use magnetisation in combination with radiofrequency (RF) pulses to obtain RF signals from nuclei of interest, usually water protons (^{1}H) due to their natural abundance and sensitivity. Briefly, protons spinning in a magnetic field are 'excited' with a targeted RF pulse. During their subsequent 'relaxation' back to their equilibrium state they emit RF, which is measured with a coil (antenna). MRI can provide information not only on the volume of gastric content fractions, but also on intra-gastric processes such as phase separation and clot formation, on gallbladder responses[15], and on intestinal parameters such as intestinal motility and small bowel water content (for overviews see Marciani *et al.*[16] and Spiller and Marciani[17]). We argue that by virtue of this common ground NMR measurements of *in vitro* samples or gastric aspirates can be used to aid the interpretation of substance-specific MR characteristics such as signal relaxation rates in a digestion context. This may serve to inform and validate MRI measurements of the same *in vitro* system as well as an *in vivo* equivalent, which in turn can validate and inform *in vitro* digestion models (Fig. 1). Thus, this review outlines the current use of MR techniques to study digestion and highlights their potential for providing markers of digestive processes such as gastric coagulation and nutrient breakdown and how MR techniques in combination with other measures may bridge the gap between *in vitro* and *in vivo* research. Collectively, insights from such interdisciplinary studies can foster the optimisation of food properties for different applications in health and disease.

In vitro digestion models

To study digestion, various *in vitro* digestion models that can be used to mimic one or more digestion phases, such as gastric or intestinal digestion, have been developed[18]. These models can also be applied to study absorption of the digested material, by incorporating intestinal cell cultures[19]. They vary from simple static models to highly sophisticated dynamic, computer-controlled gastrointestinal models[20]. In static models, the digestive fluid and food materials are constant, and hence they are convenient for investigating the mechanisms of mass transport and structure breakdown[7,21,22]. Dynamic models, such as the Tim models[23] and the SHIME model[24], include factors such as gastric emptying[25] (GE) and inflow of gastric and intestinal juice. Therefore, such models are more physiologically accurate than static models.

With the use of *in vitro* models, multiple approaches can be combined to determine the progression of digestion by e.g. (bio-)chemical and physical analysis of digesta samples. Several chemical analysis approaches have been applied to measure food hydrolysis during digestion. For example, examining changes in the size of peptides or amount of free amino groups for protein digestion[9,26], glucose for starch digestion[27] and NEFA for fat digestion[28]. From a physical perspective, rheology or texture analysis, sometimes combined with microscopy, are used to measure changes in physical

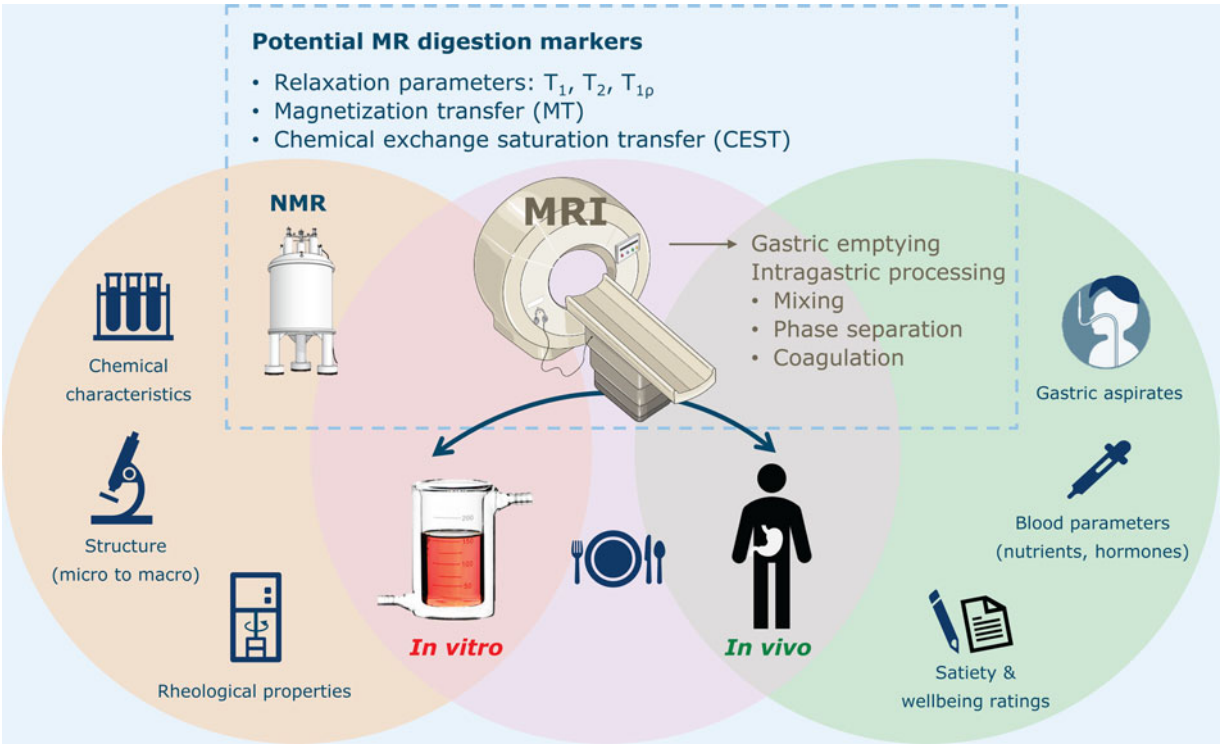

Fig. 1. (Colour online) Overview of the proposed interdisciplinary approach to study digestion by employing magnetic resonance (MR) techniques in combination with a variety of other measurements.

properties such as viscosity, or in the structure of food particles (from macro to micro) during digestion[29–31]. Recently, several other approaches have been used to study digestion, e.g. hyperspectral imaging to monitor the mass transfer between digestive fluid and food particles[32] and MR techniques to monitor the hydrolysis of nutrients and changes in food composition (Bordoni *et al.*[33]; Deng *et al.*[26]).

The advantages of using *in vitro* models include easy sampling, well controlled and reproducible conditions, ability to assess chemical processes in detail, and the absence of ethical restrictions. In addition, such simplified systems also make interpretation easier (within model boundaries) and multiple follow-up experiments can be readily done to further unravel observed phenomena. However, the validation of *in vitro* models remains a big challenge due to the inherent simplifications such as the absence of feedback mechanisms. For instance, secretion of digestive juices in response to a meal *in vivo* is regulated by the autonomic nervous system and several hormones, which is extremely challenging to replicate within *in vitro* models[8]. To aid the validation of *in vitro* models, it is of interest to investigate the potential of non-invasive approaches for *in vivo* monitoring of digestion. Several promising MR techniques are described in the following sections.

NMR spectroscopy

NMR spectroscopy is a non-destructive technique often used in the fields of biology, chemistry and food technology to determine the molecular structure and to quantify the concentration of molecules in a sample. With NMR the interactions between an external magnetic field and atomic nuclei that have a magnetic property can be observed. The main nucleus of interest in NMR is a proton (^{1}H). Examples of other commonly utilised nuclei in NMR are ^{13}C, ^{31}P and ^{15}N. In a typical NMR measurement, an NMR spectrum is recorded in which the position of the peaks can provide information on the molecular structure of the compounds present in the sample. Furthermore, the area under the curve of NMR peaks is proportional to the number of nuclei giving rise to the peak[14,34]. Therefore, it is possible to estimate (changes in) the molar percentage or concentration of several components. The combination of molecular level and quantitative information that can be obtained with NMR makes it a promising technique for examining food digestion *in vitro*. Specifically, it may be used to monitor macronutrient hydrolysis. For example, NMR has been used to quantify the products of lipid hydrolysis, such as diglycerides and fatty acids, in complex lipid mixtures[35] and in foods, such as fish[36,37] and sunflower oil[38] during *in vitro* digestion. In addition to lipid hydrolysis studies, NMR has also been applied to study protein hydrolysis *in vitro*. Sundekilde *et al.*[39] used NMR for monitoring enzyme-assisted hydrolysis of animal proteins under real-time conditions directly in the NMR spectrometer. This approach enabled the monitoring of free amino acids produced during enzymatic hydrolysis. Bordoni *et al.*[33] used NMR to monitor digestion of cheese in an *in vitro* digestion model simulating digestion in the mouth, stomach and small intestine.

Also, NMR spectroscopy is capable of providing information on the kinetics of carbohydrate hydrolysis under acidic conditions[40,41]. While these applications show that NMR spectroscopy is a powerful tool for monitoring food digestion *in vitro*, it is less suitable for *in vivo* studies. NMR spectra from *in vivo* samples will be more complex and more difficult to interpret. Moreover, NMR only allows the detection of molecules that are present in sufficiently high concentrations, which may not be the case *in vivo*.

NMR relaxometry

In addition to NMR spectroscopy, there are several other MR techniques that could be used for both *in vitro* and *in vivo* monitoring of different aspects of food digestion. These include measurement of T_1 and T_2 relaxation times and chemical exchange markers with NMR or MRI (Fig. 1).

T_1 and T_2 relaxation times

T_1 and T_2 relaxation times reflect how protons in a magnetic field relax back to their equilibrium position after excitation by an RF pulse. The main applications of T_1 and T_2 are based on the investigation of the relaxation behaviour of water protons in different environments[42]. Water proton relaxation is mainly determined by their mobility and is affected by macromolecular composition and structure. T_1 and T_2 measurements have been used to study various food properties such as moisture content, food structure and macromolecule concentration[43]. For instance, Ziegler *et al.*[44] used T_1 measurements to predict water migration in starch-pectin gels during drying since T_1 decreases with the decrease of their moisture content. T_2 was used to predict water-holding capacity of whey protein particles; a higher water-holding capacity is associated with a longer T_2. Similarly, T_2 has been used to study the swelling of hydrogels[45,46]. T_2 has also been used to study the local structure of cheese; due to the inhomogeneity of the cheese, three distinct T_2 relaxation components could be identified reflecting serum water (the water accumulated in the protein network), the water inside meshes of the casein gel-like network, and the water trapped within the casein matrix[47]. In addition, T_2 can be used to determine the protein concentration in casein solutions; with increasing concentration the T_2 decreases[48]. These examples show that T_1 and T_2 can be used to monitor changes in water migration, food structure and the composition of food and digestive juice that take place during digestion[6]. Despite this, T_1 and T_2 measurements have only been applied in a limited number of digestion studies. For instance, T_2 has been shown to be useful in detecting penetration of digestion fluid into the food matrix during *in vitro* digestion[33,49]. Another study showed a linear association between viscosity of locust bean gum meal and T_2 *in vitro*, and highlighted the possible application of T_2 to monitor changes in meal viscosity in the gastric lumen *in vivo* with the use of MRI[50]. Moreover, in our recent study we show that the hydrolysis of protein during *in vitro* gastric digestion can be monitored by T_2; T_2 was associated with protein released from food particles into the surrounding liquid[51]. However, *in vivo* gastric digestion is more complicated than the static *in vitro* model used here, which e.g. does not take into account dynamic processes such as the production of gastric juice and GE. These processes will introduce changes in the system that have multiple effects such as pH changes and dilution, in addition to the hydrolysis of nutrients. Because T_1 and T_2 are affected by many such factors, careful validation is needed to be able to interpret changes in T_1 and T_2 in different digestion contexts. This requires further investigation under dynamic circumstances, both *in vitro* and *in vivo* and the combination of NMR and MRI T_1 and T_2 measurements. For example, as shown in Fig. 2, the NMR T_2 spectrum shows separate peaks that represent the protein gel and the simulated gastric fluid around it. This information contributes to the interpretation of the MRI T_2 maps.

$T_{1\rho}$

Another relaxation time of interest in MR is the $T_{1\rho}$ or the T_1 relaxation time in the rotating frame. $T_{1\rho}$ is useful to study low-frequency motion processes and chemical exchange in biological tissues. It is measured by applying an additional RF pulse after the excitation pulse to lock the magnetisation in the rotating frame. The time it takes for the locked magnetisation to decay to zero is the $T_{1\rho}$ relaxation time[52]. In addition to conventional relaxation time measurements, T_2 and $T_{1\rho}$ relaxation time dispersion measurements[53] are promising MR markers for monitoring digestion because they can be used to examine macromolecules in solution or the interaction between bulk water protons and exchangeable macromolecule protons in semi-solids. In relaxation time dispersion measurements, the relaxation time is measured under varying measurement conditions. In the presence of chemical exchange, for example proton exchange between a macromolecule and bulk water, a dispersion of the relaxation time under those varying conditions can be observed. The extent of dispersion depends on the rate of chemical exchange. Compared to conventional relaxation time measurements, relaxation dispersion is more quantitative, since the experimental data can be fitted with theoretical models of two- or three-site exchange from which the exchange rate can be extracted[52,54,55]. This exchange rate depends on the state of a macromolecule, e.g. intact or digested, and hence, can potentially be related to the kinetics of digestion. However, to date the application of T_2 dispersion has been limited to investigating molecular dynamics of proteins *in vitro*[56]. $T_{1\rho}$ dispersion, in contrast, has been more commonly applied in *in vivo* MRI studies, but not yet in the domain of digestion. Duvvuri *et al.*[57] suggested that in cartilage proton exchange between protons from NH and OH groups in the proteoglycans and water dominate the $T_{1\rho}$ dispersion of water. They showed that the exchange rates increase with proteoglycan breakdown. This suggests that $T_{1\rho}$

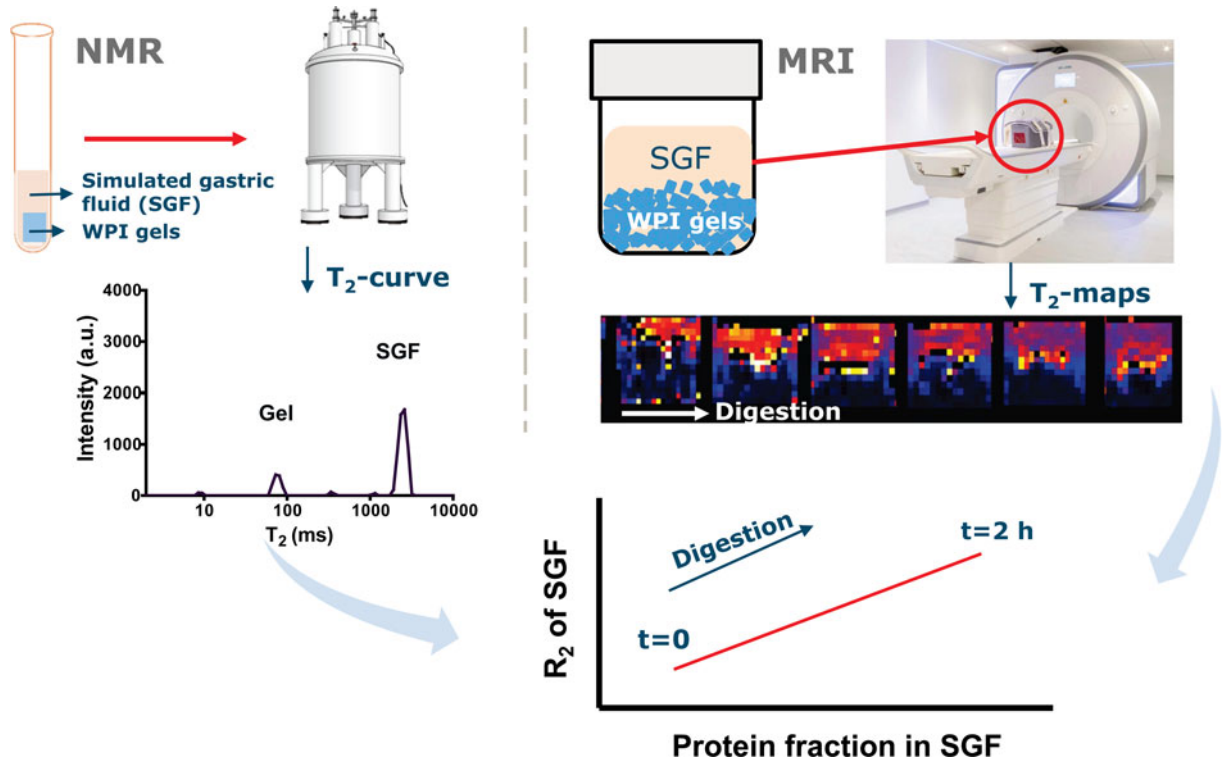

Fig. 2. (Colour online) Illustration of how NMR and MRI of the same *in vitro* model can be used to study the dependence of magnetic resonance parameters on nutrient breakdown. Shown here is the increase in T_2 relaxation rate ($R_2 = 1/T_2$), measured with either technique, and the protein fraction in simulated gastric juice (SGF) during digestion of whey protein gel pieces. Adapted from Deng *et al.*[51].

dispersion has potential as a marker for *in vivo* monitoring of protein digestion.

In conclusion, MR relaxation parameters and their meaning in a digestion context need to be further elucidated. NMR can aid the interpretation of *in vitro* and *in vivo* relaxometry measurements with MRI. In turn, *in vivo* MRI can serve to validate and inform *in vitro* models.

NMR cross-relaxation

Magnetisation transfer

Magnetisation transfer (MT) is an MR technique that is used to create a contrast between tissues in which protons are present in three different states: (i) in free water, (ii) bound to semi-solid macromolecules and (iii) as water in the hydration layer between the macromolecules and free water. In an MT measurement, the magnetisation of macromolecular protons is saturated by application of an RF pulse. The saturation is then transferred to protons in water through proton exchange resulting in a decrease in the water signal intensity. The MT rate is sensitive to the formation of a semi-solid structure[58] and hence could serve as a marker for the degree of coagulation of proteins during gastric digestion. The degree of coagulation can affect digestion and GE rate[59].

Chemical exchange saturation transfer

Chemical exchange saturation transfer (CEST) is a relatively novel MR technique. Its principle is similar to that of MT where saturated protons are exchanged with non-saturated protons in water. However, the main difference is that in CEST, saturation transfer takes place between a (macro)molecule in solution and water and that the saturation is frequency-selective[60]. Dona *et al.*[41] showed that CEST can be used to monitor the *in vitro* enzymatic degradation of macromolecular starch granules. Moreover, from their CEST measurement, the kinetics of glucose release during the enzymatic hydrolysis of cooked starch was successfully monitored, demonstrating the potential of CEST for obtaining quantitative information on food digestion[41]. Longo *et al.*[61] used CEST to monitor the aggregation of bovine serum albumin during heat treatment and its subsequent hydrolysis by a protease *in vitro*. During heat treatment the proteins aggregate, thereby decreasing the accessibility of the protein protons for exchange with water protons. However, after digestion of the heat-treated protein, the protons become accessible for exchange again, resulting in an increase in saturation transfer. This suggests that CEST can serve as a marker for monitoring protein digestion and how this is affected by heat treatments. Another interesting application of CEST is pH imaging in which the endogenous amide proton transfer rate is related to the pH in the tissue of interest[62]. Most applications of CEST pH imaging are done in the brain[63–65] where the pH is between 6·5 and 8·5. It would be of great utility if CEST could be used to make 3-D stomach pH maps since pH is an important factor that among others influences pepsin activity. However, stomach pH is

between 1·5 and 3·5 in children or adults and between 3·5 and 5·5 in infants. This renders stomach pH imaging challenging due to the slower amide proton transfer rate at low pH.

Magnetic resonance relaxometry and cross-relaxation outlook

While NMR/MRI relaxometry and cross-relaxation techniques have not been applied much in food digestion research, the applications to date suggest that they have potential for monitoring digestion. For *in vitro* studies, it is possible to obtain quantitative information, such as the proton exchange rate from relaxation time dispersion, MT or CEST data by fitting with biophysical models of two- or three-site chemical exchange. The chemical exchange rate is expected to depend on the pH, macromolecular concentration and semi-solid fraction, which are all factors that change during digestion. However, reliable modelling of such data can only be done on sufficiently large data sets. The collection of such data sets is time-consuming and limited to *in vitro* studies. The main challenge lies in optimising experimental conditions such as the measurement time, size of datasets and sub-set of fitting parameters to enable quantitative application of the same measurements *in vivo*. Moreover, *in vivo* data acquisition has practical constraints and interpretation is harder. There are limits to the time that volunteers can spend in the scanner and scans have to be made during breath hold or with respiratory triggering minimise image artefacts caused by breathing movements. Physiological noise and constraints on acquisition times will result in poorer data quality compared to *in vitro* data. However, these are common challenges for any *in vivo* application of MRI, and there is continuing technical development aimed at ameliorating these issues. Moreover, as pointed out before, careful *in vitro* validation experiments can be used in the development and validation of these promising MR markers of digestion.

MRI

MRI is a popular medical imaging technique because it does not use ionising radiation and is extremely versatile. Similar to in NMR, in MRI a strong magnetic field (1·5–7 T) is used to line up water protons (^{1}H) in the body. These protons are energised by exposing them to specific RF pulses. When they relax back into their lower-energy state RF is emitted. This RF signal (echo) is measured with a coil (antenna). By varying the local magnetic field, RF pulse characteristics and the timing of RF measurement it is possible to reconstruct different types of images from the measured RF signals[66]. Among other factors, the contrast of these images depends on the local T_1 and T_2 relaxation rates, which vary between tissue types. The anatomical detail provided by MRI allows accurate visualisation of the stomach and its contents[67]. Also, because MRI scan sequences can be configured to be sensitive to different aspects of gastric or intestinal contents, MRI is suitable for investigating complex meals and intragastric processes such as gastric sieving, phase separation and coagulation (see later). Moreover, MRI can be used to examine model systems, such as *in vitro* digestion models, as well as human subjects using the same scan sequences (Fig. 1). This makes it an excellent technique to bridge *in vitro* and *in vivo* research.

In vivo MRI: gastric contents

Since the 1990s, gastric digestion research has embraced MRI as a method to measure GE, i.e. the change in gastric content volume over time in human subjects[68,69]. Understanding GE is important since it plays a major role in digestion, satiety and nutrient absorption. GE is a rate-limiting step in the delivery of nutrients to the small intestine for further break-down, and one of the factors influencing the susceptibility to maldigestion[22]. GE rate is largely determined by the chemical characteristics of food, mainly the macronutrient content, but also physical characteristics, such as the viscosity[70,71]. There are various other techniques to assess GE rate, but the most common approaches are either indirect (C-isotope breath analysis and paracetamol absorption test), or involve the use of ionising radiation (gamma scintigraphy[72]). Advantages of MRI are that it is well suited for individual GE assessment and less dependent on the food matrix than indirect tracer-based methods such as C-isotope breath analysis[73]. Quantification of postprandial volume changes with MRI has been shown to have low inter-observer variability, unless the stomach is nearly empty[74].

GE can be different for different food fractions. So-called, 'gastric sieving' happens when a meal consists of multiple textures, or when these arise as part of digestion; the (more) liquid component has been shown to quickly 'sieve' through the stomach while the more solid part of the meal is retained[75–77]. When the two components are blended into a (nutrient-rich) liquid, satiety is enhanced because the fast entry of energy into the duodenum slows down GE[77]. Similar sieving has been shown to occur for water and a meal shake; water can drain from the stomach while a layer of energetic liquid is retained[78]. For such foods and mechanisms tracer-based methods are not very suitable, since different tracers with different kinetics would be required to show the transit of watery and fatty components separately (see e.g. Collins *et al.*[75] for a double isotope approach), depending on their solubility.

MRI can also show air or gas volumes in the stomach, which can significantly affect perceived fullness and appetite through providing gastric distention. For example, aerated milk-based drinks (foams) increased gastric volume and reduced hunger more than an isoenergetic liquid control drink[79]. This underscores the notion that stretching of the stomach wall contributes to satiation and perceived fullness. In a well-controlled study which used MRI in combination with measurement of gastric pressure Kwiatek *et al.*[80] elegantly show that there is a distinct early phase of GE with relatively rapid, uncontrolled passage of nutrients into the

duodenum, which is faster for larger meal volumes and unaffected by energetic load. However, subsequently the delivery of nutrients to the duodenum is related to the overall energy load of the meal, i.e. GE is inhibited more for greater energetic loads[80]. Subsequent work of our group with 500-ml meal shakes confirmed that energy density is the main driver of GE, but that greater viscosity additionally slows GE, and is more important for perceived fullness than gastric content volume[71]. Similarly, a semi-solid liquid meal had lower GE rate and resulted in greater suppression of appetite over 3 h than an iso-energetic liquid meal, despite lower plasma cholecystokinin (a satiety-related hormone) release in the first hour[81]. Appetite was correlated with gastric content volume rather than GE rate or plasma cholecystokinin, which suggests that the longer gastric retention was driving the decrease in appetite[81]. These studies illustrate the usefulness of MRI to study the gastric behaviour of different foods and drinks at a macroscopic level, especially in combination with other physiological measures and subjective ratings.

In vivo MRI: intra-gastric processes

In addition to the processes discussed earlier, MRI is very suitable to investigate other intragastric processes such as layering (phase separation), mixing and macroscopic changes in the texture of the chyme, such as gelling or coagulation, because these will cause changes in the T_1 and T_2 relaxation rates and can thus be visualised. These processes depend on multiple food properties which are collectively referred to as the food matrix, which has been framed as 'a physical domain that contains and/or interacts with specific constituents of a food (e.g. a nutrient) providing functionalities and behaviours which are different from those exhibited by the components in isolation or a free state'[82]. The food matrix plays a key role in the kinetics of transit and hydrolysis of macronutrients[82].

Layering and fat quantification

In the case of layer formation (phase separation), the different layers can simply be quantified on an MRI image. For example, when a more fatty layer forms this appears darker on a T_2-weighted gastric MRI image than a more watery layer, see e.g.[78]. Similarly, in the first hour after consumption breakfast porridges showed clear phase separation, with a brighter layer on top, consistent with a more liquid phase in the type of moderately T_2-weighted MRI images made, and a darker layer at the bottom, consistent with thicker or more particulate material[83].

So far, we have mainly discussed volume measurements of different food fractions. However, after careful *in vitro* validation quantitative MRI measurements can be performed *in vivo*. For example, Marciani *et al.* investigated the dilution of polysaccharide test meals by gastric secretions[70] by exploiting the association between the T_2 relaxation time (T_2) and polysaccharide concentration[50]. MRI is also well-suited to distinguish water and fat. Kunz *et al.*[84] specifically measured the fat component of a pasta meal (mayonnaise). By first assessing *in vitro* samples in which the fat concentration was varied they were able to calibrate their *in vivo* MRI measurements. Liu *et al.*[85] studied gastric and duodenal fat emptying and emulsion processing (creaming and phase separation) using fat emulsions that were administered through a nasogastric tube. They not only calibrated their fat quantification approach with *in vitro* MRI, but they also took gastric aspirate samples for further validation *in vivo*. The resulting fat fraction maps and intragastric emulsion profiles showed details of intraluminal phase separation and creaming that were not (well) visible on the conventional MRI images[85]. This approach was taken further by Scheuble *et al.*[86] who studied the gastric behaviour of fat emulsions stabilised by three different biopolymers *in vitro* as well as *in vivo* with MRI, combined with blood sampling for measurement of TAG and cholecystokinin concentrations. These studies on fat digestion showcase that MRI can bridge the gap between *in vitro* digestion models and *in vivo* behaviour by carefully combining different types of measurements.

Coagulation

While eventual breakdown of structure is necessary to allow for GE, gastric conditions can also induce changes in the texture of the chyme such as gelling and coagulation. Since this involves the transformation from liquid to (semi-)solid it could slow GE. While coagulation will be readily visible on conventional stomach MRI images (see Fig. 3) it has hardly been systematically quantified. Coletta *et al.*[87] looked at GE of breads with different gluten contents and additionally visually categorised the degree of heterogeneity of the food bolus in the stomach on MRI scans. They found that gluten did not change GE, although it made the chyme more heterogeneous. Also, there were no differences between the breads in gastrointestinal symptoms, postprandial small bowel water content, colonic volume and gas content measured with MRI. Another example is milk protein coagulation; protein digestion is strongly affected by pH changes in the stomach and the associated activity of pepsin. Digestion by pepsin as well as the pH decline over time cause the caseins in milk to coagulate as demonstrated *in vitro*[88–90], while the whey protein remains soluble. Casein coagulation is believed to slow down GE; the stomach empties only particles into the small intestine if they have a size of 1–2 mm[91]. This notion is supported by *in vivo* studies showing that amino acids from whey protein appear faster in the blood than those from casein[92,93]. *In vitro* data also show that casein coagulation is affected by several factors such as processing-induced protein modifications, product composition such as mineral composition, and variations in gastric acidification and protease secretion[94,95]. In addition, the source of the protein may influence gastric coagulation[96]. However, these findings require verification *in vivo*. This would benefit from accurate quantification of chyme structure changes. Although visual grading is a useful and relatively simple approach, this could be taken further by validating the use of image texture metrics that can capture the observed

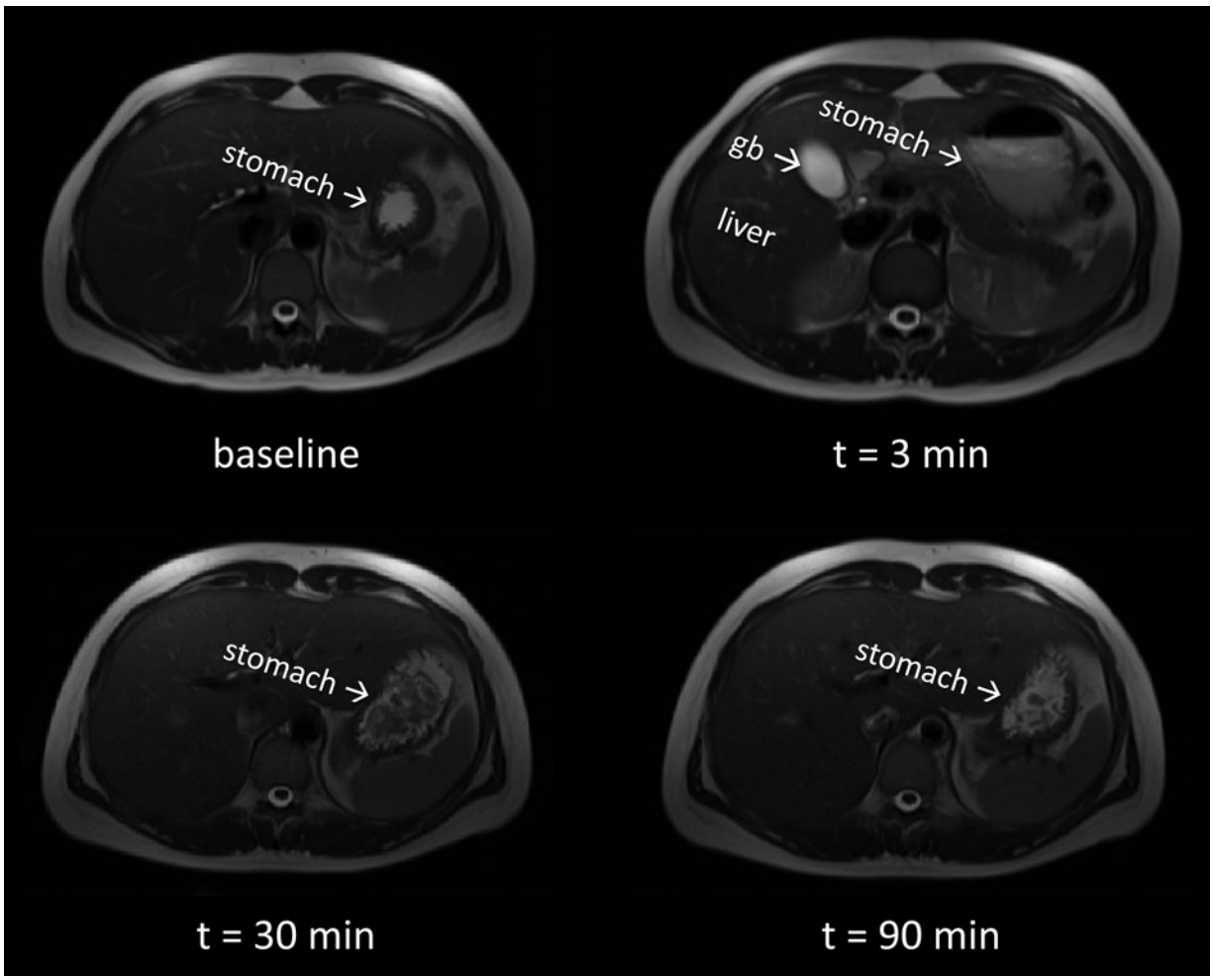

Fig. 3. Examples of T_2-weighted magnetic resonance images showing cross-sections through an empty stomach after an overnight fast (baseline) and after 250 ml milk consumption. At $t = 3$ min the gallbladder (gb) is clearly visible, and the gastric contents visible at baseline can be seen on top of the milk. At $t = 30$ and 90 min milk protein coagulation can be observed.

heterogeneity; this may provide a more sensitive and objective assessment and information on what a certain degree of heterogeneity in an MRI image reflects in terms of particle sizes and texture attributes of coagulates. Such food matrix/chyme characteristics may influence gastric digestion and subsequent intestinal digestion and bioavailability (see e.g. Fardet *et al.*[97]).

Blood parameters

Although it is quite well possible to obtain blood samples from participants lying in an MRI scanner, not many studies have combined MRI of the digestive tract with blood sampling to assess hormone responses related to digestion and nutrient bioavailability (see e.g. Alyami *et al.*[83] and Mackie *et al.*[81]). GE and more detailed MRI markers of digestion could be linked to *in vivo* nutrient bioavailability measures such as blood glucose, fatty acid and amino acid profiles. For example, *in vitro* work showing that GE rate affects protein digestion, amino acid absorption and subsequent whole body protein anabolism after a meal[98] could be validated with such an approach. One current candidate MRI marker for measuring the breakdown of protein foods is the T_2 of the surrounding gastric fluid[51], but as discussed earlier other MRI measures may also be of interest. Such studies would provide unprecedented detail on how food characteristics affect digestion and bioavailability and can inform optimised food design. For example, the effects of different protein sources and processing-induced protein modification on GE, protein digestion and amino acid absorption measured by MRI and amino acid absorption in the blood could be explored. Better understanding of the determinants of protein digestion can inform choices for or design of products that are easier digestible, which is beneficial for people who have trouble ingesting enough protein, such as older adults, athletes and critically ill.

Conclusion

In addition to more macroscopic structural information, NMR and MRI have the potential to provide molecular-

level and quantitative information on *in vivo* gastric (protein) digestion. This requires careful validation in order to understand what a specific MR parameter, or set of parameters, means in a specific digestion context. The resulting MR markers of digestion can be used to validate and inform *in vitro* digestion models and may bridge the gap between *in vitro* and *in vivo* studies. This can aid the optimisation of food properties for different applications in health and disease.

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Conflict of Interest

None.

Authorship

The authors jointly wrote and approved the paper. P. A. M. S. had final responsibility for the content.

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