Challenges in Quantifying Digestion

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1 Introduction

Eating habits are continuously changing, which is often related to new foods and food ingredients on the market. For example, due to the increasing demand for proteins, new sources of proteins are introduced on the market, such as proteins from insects, algae, and fish industry by-products. Another aspect that drives the production of new food products is new insights in nutritional quality in general or related to specific age groups, such as infants, the elderly, and people with disease conditions.

These changes give a continuous need for in vivo and in vitro studies to determine the quality of food products in terms of, among others, palatability, digestibility, and bioavailability of nutrients and/or functional compounds.

In this section we describe the challenges in qualifying digestion of food products and the bioavailability of nutrients and functional compounds in human and animal studies as well as in in vitro studies.

2 Challenges in Terminology

One of the first challenges is: do we speak the same language in food and nutrition research? It is important that the scientists in this field have the same perception and understanding of the terminology. So it is essential to use a standard type of "professional language" with uniform terminology and definitions. Different organizations have published guidelines on definitions and terminology. For example, the

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European Society for Clinical Nutrition and Metabolism (ESPEN; www.espen.org/education/espen-guidelines) appointed a Terminology Consensus Group in the field of clinical nutrition (Cederholm et al., 2017). Individual authors or institutes also publish research papers focused on stimulation of uniform terminology. In relation to digestion and bioavailability of food compounds it is important to have identical and consistent terminology, not only for terms as "in vivo bioavailability" versus "in vitro bioaccessibility" of nutrients (Fernandez-Garcia, Carvajal-Lerida, & Perez-Galvez, 2009), but also for definitions related to food compounds, such as "trans-fatty acids" (Wang & Proctor, 2013) and "dietary fiber" (Macagnan, Da Silva, & Hecktheuer, 2016; Miller Jones, 2014).

It is crucial to check these different guidelines on terminology and definitions for the specific food products and/or nutrients. We ought to use consequently the same terminology as far as possible or at least mention the origin of the definition used in the food digestion experiments.

3 Challenges in Analysis

Once we know which compounds are defined within a "definition" the next challenge is the standardization of the extraction and analytical method. Different organizations are involved in standardization of (bio)chemical analysis methods, such as the Food and Agriculture Organization (FAO) of the United Nations (www.fao.org/publications/en/) on food analysis in general and on food energy methods of analysis and conversion factors (FAO, 2003), the Association of Official Analytical Chemists (today: Communities; AOAC; www.aoac.org), and the European Commission for functional food ingredients (Buchgraber & Karaali, 2005). These organizations give information on (globally accepted) standardized analytical methods, including nutrients and active food ingredients. Approved methods related to specific food products are also available, such as those introduced by the American Association of Cereal Chemists (AACC[1] International; www.aaccnet.org). They offer descriptions of analytical methods for a broad variety of food compounds in cereal grains. The use of these approved methods in digestion studies will contribute to the standardization of experimental results.

To evaluate the quality of the analytical methods as used in your lab, it is possible to use reference materials for the calibration of your analysis instruments and to improve the reliability of the analytical results. Via the Institute for Reference Materials and Measurements (IRMM, ec.europa.eu/jrc/en/reference-materials) a catalogue with 800 different certified reference materials is freely available (IRMM, 2015).

[1] AACC also stands for American Association for Clinical Chemistry; a global scientific and medical professional organization dedicated to clinical laboratory science and its application to health care (www.aacc.org).

In case you want to validate your analytical method in a collaborative study, the AOAC International has guidelines available for the setup of these types of collaborative studies (AOAC International, 1995).

4 Challenges in Human Digestion Studies

Human clinical studies may be regarded as "the gold standard" for food digestion research. However, the performance of a human clinical study is a real challenge. For human intervention studies, to evaluate food digestion and quality, there are different general guidelines available, such as "scientific standards" for intervention trials and good clinical practice (GCP) by Woodside, Koletzko, Patterson, and Welch (2013) and Schmitt et al. (2012) or for evaluating health benefits of foods by Welch et al. (2011). These guidelines are mostly based on consensus by expert groups (e.g., ILSI Europe (Brussels, Belgium; ilsi.eu/task-forces/nutrition/)). Sometimes they are based on a review of methodologies, such as for analyzing the glycemic index in humans on the intake of carbohydrates by Brouns et al. (2005) and on energy metabolism in humans by Lam and Ravussin (2016).

A complicating factor in digestion experiments in humans is to follow exactly the digestion and bioavailability of a nutrient after oral intake. One of the techniques is the use of food compounds intrinsically labeled with stable isotopes, such as fatty acids (Ecker & Liebisch, 2014), proteins (Geboes et al., 2004), minerals (Abrams, 2003), or vitamins such as dietary carotenoids (Van Lieshout, West, & Van Breemen, 2003). Nevertheless, the collection of samples from human intervention studies is limited to, for example, blood, urine, and fecal samples. This may hamper the outcome of the studies.

The consequences of these intervention studies are that these studies are extremely expensive and time-consuming. Moreover, the pressure on ethical aspects is increasing, due to the rules for liability and corporate social responsibility. Based on the ethical principles for medical research involving human subjects (known as the Declaration of Helsinki) by the World Medical Association (www.wma.net/what-we-do/medical-ethics/), many (governmental) organizations have defined ethical rules for human intervention studies, such as the National Institute of Health (bioethics.nih.gov) and World Health Organization (who.int/ethics/research/en). These rules should at least be fulfilled for grant applications.

5 Challenges in Animal Digestion Studies

Animal studies are used already for a long time as model for human nutrition studies (Baker, 2008; Gallaher, 1992; Lovegrove, Hodson, Sharma, & Lanham-New, 2015), including neonatal nutrition (Puiman & Stoll, 2008), as animal models have species-specific possibilities and limitations. On the one hand, specific non-invasive

techniques such as the ^{13}C-labeled breath test (McCue & Welch, 2016) and invasive techniques such as fistulation (Swindle, Smith, & Goodrich, 1998), are available for animal studies, with legislative and ethical restrictions. On the other hand, there are challenges in the extrapolation of results to the human situation. For food digestion studies (e.g., protein quality assessment) pigs and rats are advised as animal models (FAO, 2013). However, it was found that the true ileal protein and amino acid digestibility in pigs was significantly lower than that in humans (Deglaire, Bos, Tomé, & Moughan, 2009; Rowan, Moughan, Wilson, Maher, & Tasman-Jones, 1994). The predictive quality of digestion experiment in rats showed a correlation coefficient of only 0.46 (Bodwell, Satterlee, & Hackler, 1980). The reason for discrepancy between results from human versus animal studies is the difference in gastrointestinal physiology. For example, the gastric pH and gastric emptying time can be drastically different between animal species and humans. This makes the selection of the animal species for digestion studies and the interpretation of results a real challenge (e.g., Fuller & Tomé, 2005).

Laboratory animals are also protected by legislation and guidelines, in Europe for example by Directive 2010/63/EU (http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm) and in USA by NIH guidelines (8th edition, 2010; https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals_prepub.pdf). In the UK they developed guidelines to improve the reporting of research using animals, aiming to maximize the published information and to minimize unnecessary animal studies (Animal Research: Report of In Vivo Experiments; ARRIVE; https://www.nc3rs.org.uk/arrive-guidelines).

6 Challenges in In Vitro Digestion Studies

In vitro digestion studies have been and still are performed in a broad range of digestion methods and models, from simple static beaker experiments (Babinszky, Van der Meer, Boer, & den Hartog, 1990) to highly sophisticated dynamic, computer-controlled gastrointestinal models (Bellmann, Lelieveld, Gorissen, Minekus, & Havenaar, 2016; Minekus, Marteau, Havenaar, & Huis in 't Veld, 1995).

Various review papers describe the differences between models and methods in relation to food digestion and measuring the availability for intestinal absorption of nutrients (bioaccessibility), such as for adults (Alminger et al., 2014; Guerra et al., 2012; Ting, Zhao, Xia, & Huang, 2015; Verhoeckx, 2015; Williams et al., 2015) and infants (Nguyen, Bhandari, Cichero, & Prakash, 2015). These differences in methods and models make the comparison between in vitro digestion experiments quite complex. Therefore, the EU project "InfoGest" tries to standardize the simulated in vitro conditions, first for the static digestion models for adults (Minekus et al., 2014) and later for dynamic in vitro models (Dupont et al., 2017). These standardizations should result in more comparable in vitro data. Regardless of the attempt to standardize static digestion methods, there was consensus about the limited predic-

tive quality of static methods due to lack of the simulation of realistic kinetic gastrointestinal conditions (Minekus et al., 2014).

To simulate the realistic conditions in the stomach and small intestine for digestion experiments, the (average) physiological kinetic conditions in the lumen of the stomach and small intestine should be "translated" to dynamic in vitro models. Many review articles describe the gastrointestinal physiology after intake of different types of meals for adults (e.g., Barros, Retamal, Torres, Zúñiga, & Troncoso, 2016; Culen, Rezacova, Jampilek, & Dohnal, 2013; Varum, Hatton, & Basit, 2013) as well as for infants (e.g., Bourlieu et al., 2014; Kamstrup, Berthelsen, Sasene, Selen, & Müllertz, 2017).

This "translation" to dynamic in vitro gastrointestinal models also has several challenges (Guerra et al., 2012). One of these challenges is the interpretation of enzyme activities, especially the pancreatic digestive enzymes (DiMagno & Layer, 1993). Different definitions of digestive enzyme activities and enzyme assays have been published, including the use of coenzymes, different substrates and pH values for digestion, such as those described for infant digestion by Abrahamse et al. (2012). The next challenge is the availability of appropriate, purified digestive enzymes. Specifically, human gastric and pancreatic enzymes as well as brush border enzymes (Picariello, Ferranti, & Addeo, 2016) are not commercially available. So alternative enzymes such as pancreatic enzymes from pigs are used based on the knowledge that the pig is the best available animal model for human digestion (Guilloteau, Zabielski, Hammon, & Metges, 2010). As alternative for gastric lipase and purified proteases, commercial enzymes of animal or microbial origin are available. They need to be selected on their physicochemical characteristics, such as activity and stability under site-specific human gastrointestinal conditions (Minekus et al., 2014). Likewise, bile is an important secretion compound for food digestion (Maldonado-Valderrama, Wilde, Macierzanka, & Mackie, 2011), facing the same challenges for in vitro models as digestive enzymes in relation to the secreted amount during the digestion process, composition of bile salts, and availability of human bile. Commercially available porcine or bovine bile is often used as an alternative to human bile (Minekus et al., 2014).

After the optimal in vitro model (hardware), settings (software), and composition of secretion fluids have been set up, the next important challenge is the validation of the in vitro digestion model. First, an operational quality (OQ) validation is necessary: does the dynamic model simulate in a controlled and reproducible way the in vivo physiological conditions? An example of such an OQ validation has been described by Bellmann et al. (2016) for the simulated conditions in the stomach in comparison to human physiological data. Second, a performance quality (PQ) validation should take place: are the in vitro results predictive for human clinical digestion studies? The challenge is how to compare in vitro bioaccessibility data with human bioavailability data, in the light of the abovementioned challenges of human clinical studies. The optimal way of PQ validation is the use of in vitro vs. in vivo studies specifically dedicated to the in vitro–in vivo comparison, such as those described by Verwei, Freidig, Havenaar, and Groten (2006) for folate and Bellmann, Minekus, Sanders, Bosgra, and Havenaar (2017) for carbohydrate digestion. In these

studies, the in vitro gastrointestinal models were used in combination with in silico modeling for optimal prediction and comparison with human bioavailability data.

In most cases this optimal way of validation is not possible. In those cases, relevant clinical human data must be found for reproducing in vitro studies. Examples of this type of validation or evaluation are protein and fat digestibility studies under infant, adult, and elderly digestive conditions (Denis et al., 2016; Fondaco et al., 2015; Gervais et al., 2009; Havenaar et al., 2016; Maathuis, Havenaar, He, & Bellmann, 2017) as well as in vitro bioaccessibility studies for minerals and vitamins (Déat et al., 2009; van Loo-Bouwman et al., 2014; Verwei et al., 2003, 2006). These evaluation studies demonstrate that digestion experiments in dynamic in vitro gastrointestinal models may have a high predictive quality for the human situation. Validated in vitro digestive models contribute to the replacement of animal studies and the cost-efficient development of new food products.

7 Conflicts of Interest

Irrespective of the type of study, the setup and performance of the experiments, the descriptions and interpretation of the results and the final conclusions should be based on scientifically sound arguments (e.g., based on a broad literature survey). It may not in any way be biased by (vested) interests that could inappropriately influence the work. Various guidelines are available to learn more about financial or personal conflicts of interest, such as that by scientific organizations (e.g., NIH; https://www.ncbi.nlm.nih.gov/ pubmed/ 21872119) and publishers (e.g., https://www.elsevier.com/conflictsofinterest; https://publishing.aip.org/authors/conflict-of-interest).

It is advised to read one of these guidelines, in fact before starting a project, but especially before writing a scientific publication. A conflicts of interest form can be downloaded from the International Committee of Medical Journal Editors (ICMJE; http://www.icmje.org/conflicts-of-interest/). This form can be filled out, saved on your computer, and be attached to the submitted manuscript.

8 Conclusion

The continuous need of results from reliable food digestion and nutritional quality studies creates the challenge to find the most optimal way of a cost-efficient and time-efficient, ethically liable experimental setup with optimal predictive quality. Although human clinical studies seem to be the gold standard, these studies are complex, expensive and have ethical constraints and therefore only applicable for single specific studies and not for routine digestion experiments. Animal models, on the other hand, may have a low extrapolative quality due to physiological differences in comparison to humans. Thus, a conscientious selection of the animal

model in relation to the aim of the study and the ethical constraints is necessary. The latest generation of dynamic in vitro gastrointestinal models makes it possible to accurately simulate the human digestive conditions, even in relation to age (infants, adults, and the elderly). The results from food digestion studies with these dynamic models show a high predictive quality for the human situation.

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