Lessons learnt from MyCyFAPP Project: Effect of cystic fibrosis factors and inherent-to-food properties on lipid digestion in foods

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ABSTRACT

Unveiling mechanisms underpinning nutrient digestion has raised interest in the field of medical sciences for their potential application in clinical treatments. In the case of Cystic Fibrosis, there was the urgent need of understanding lipolysis to establish a criterion to adjust the dose of pancreatic enzyme supplements that patients have to take to allow digestion, given the associated exocrine pancreatic insufficiency (EPI). The aim of MyCyFAPP Project was establishing an evidence-based method to adjust pancreatic enzyme replacement therapy based on understanding lipolysis mechanisms. To solve this challenge, the still unexplored field of real foods digestion had to be addressed. A static in vitro digestion model that simulated different EPI intestinal conditions was developed to conduct an extensive experimental work with 52 foods and with different doses of the enzymatic supplements. Results could explain the role of the inherent to food and host factors affecting lipolysis. In addition, the prediction of the optimal dose of enzymes for all the studied foods was tested in a clinical trial resulting in improved growth and quality of life. This review paper provides an overview of the main findings related to the new knowledge generated in the field of lipid digestion in real foods.

Keywords: in vitro digestion, lipolysis, food matrix, intestinal pH, bile salts

1. INTRODUCTION

Multidisciplinary approaches to address challenges in the treatment of diseases have almost become a must nowadays (Surkis et al., 2016). The adoption of other health-related areas of knowledge into the medical context have proved to bring complementary and supporting scientific evidence which, treated as required in some diseases, can be translated into more efficient treatments and new therapies (Smith, Baveja, Grieb, & Mashour, 2017). Potential health-allied disciplines such as food science can offer opportunities and tools never before considered neither foreseen in traditional approaches to disease management.

Some pathologies in particular are needing the knowledge and technology from the field of food science, as in the case of exocrine pancreatic insufficiency associated to cystic fibrosis (Calvo-Lerma et al., 2017). In this genetic disease, a mutation in the CFTR trans-membrane protein is dysfunctional, leading to the thick mucus secretion that affects the pancreas among other organs, impeding the secretion of pancreatic enzymes into the small intestine (Woestenenk et al., 2015). Consequently, nutrient digestion is impaired, especially regarding lipids. To palliate the disorder, pancreatic enzyme replacement therapy (PERT), consisting of the exogenous administration of pancreatic enzyme supplements, has to be followed in every meal to enable digestion (Turck et al., 2016). However, persistent steatorrhea (residual fat in faeces) and abdominal symptoms (pain, bloating, diarrhoea and constipation) are frequent consequences despite treatment with PERT (Wouthuyzen-Bakker, Bodewes & Verkade, 2011). Up to date there was no evidence-based method to adjust the dose. The ultimate consequences of unadjusted doses, and therefore sub-optimal lipid digestion, are growth and weight stunting in the long run, which are the parameters directly correlated with disease prognosis and survival (Elborn, 2016).

Reasonably, the lack of a method was justified by the fact that the enzymatic supplements are taken daily, several times a day, and the standard parameter assessing the effectiveness/adequacy of the dose (residual faecal fat) is assessed at a specific point (Woestenenk et al., 2015), thus it is not representative from the long-term use of the supplements on a daily basis. In addition, the diet varies everyday and from one meal to another (Calvo-Lerma et al., 2017). Therefore, it is rational to assume that the dose will have to be different depending on the type of meal. This assumption leads to the concept that a specific dose of enzyme supplements should be advised for every type of food, supporting the real challenge this entails.

However, lipid digestion is a multi-factor phenomenon, as it depends on the amount and type of lipid, the structural properties of the food matrix in which lipids are contained, the gastrointestinal conditions of the individual, and of course, the dose of the enzymatic supplement (Calvo-Lerma, Fornés-Ferrer, Heredia & Andrés, 2019). Therefore, all these factors would need to be considered in order to find the optimal dose of enzymes for specific foods, considering the gastrointestinal conditions occurring in pancreatic insufficiency linked to cystic fibrosis (**Figure 1**) (Calvo-Lerma et al., 2019 a).

In this sense, in vitro digestion models are an appropriate tool to address the study of lipid digestibility, as they allow for the simulation of the gastrointestinal conditions in lab and to assess the processes and reactions underlying lipolysis (Ménard et al., 2014). In fact, some studies addressing lipolysis in real foods have already proven that both food structure and the intestinal environment conditions can really determine the fate of lipolysis (Calvo-Lerma, Fornés-Ferrer, Heredia & Andrés, 2018). These studies have unveiled some mechanisms occurring intra-luminal related to food characteristics, determining the efficacy of the pancreatic enzyme supplement. Thus, a solid hypothesis is driven: if food characteristics determine the extent of lipolysis during digestion, the

efficacy of pancreatic enzyme supplements used in PERT depends on the relation between enzyme doses and type of food intake; so that, an evidence-based method to optimally adjust the dose of enzymatic supplement can rely on the study of food digestion.

To address this hypothesis, MyCyFAPP Project, integrated by a multidisciplinary team (food technologists, food engineers, dieticians, medical doctors, psychologists, software developers and telecommunication engineers), was set up with an ultimate goal: developing an evidence-based method to adjust the dose of enzymes used in PERT (Calvo-Lerma et al., 2017). To achieve the goal, a great number of experiments were conducted in the laboratory, in which both the food characteristics and the host-related factors were analysed regarding their role on lipolysis and need of enzyme doses, on the basis of a wide range of foods and doses of the supplements.

This paper provides a summary of the strategies, tools, main findings, and their application to the real life to improve lipid digestion in patients with cystic fibrosis.

2. SET UP OF AN IN VITRO DIGESTION MODEL TO ELUCIDATE THE OPTIMAL DOSES OF PANCREATIC ENZYMES FOR PATIENTS WITH CF

Studying lipid digestion of food is a broad topic, so in order to follow an approach with a direct translational impact, knowing dietary habits of the population helps encompassing the target. A study assessing the dietary pattern of European children and adolescents with cystic fibrosis was used as the basis to find the fat-containing foods contributing the most to daily lipid intake (Calvo-Lerma et al., 2019 b). This study showed that the focus should be placed on dairy products (milk, cheese, yoghurt and desserts), meat, fish, eggs, chocolate, pastries, bakery and nuts. A total of 52 foods were finally selected for the in vitro digestion studies (**Figure 2A**).

Then, an in vitro digestion model for CF patients was needed to address lipolysis studies in the selected foods. Given the large number of experiments to be performed in several foods, a static in vitro digestion model was targeted, as it allows for screening several experimental conditions simultaneously and drive results that are generated following the same methodology (Minekus et al., 2014). The international protocol proposed by Minekus et al. (2014) supported the simulation of the stock solutions for the digestive fluids and the oral, gastric and intestinal digestion times. To this model, the necessary amendments were applied to simulate the specific intestinal conditions occurring in EPI and cystic fibrosis: use of pancreatic enzyme supplements instead of pancreatin, lower bile salts concentration up to 1 mM and lower intestinal pH (pH 6). These amendments were supported by the clinical partners involved in MyCyFAPP and a thorough literature research in which the alterations occurring in this pathology were described: the obstruction of the pancreatic duct impedes the secretion of the pancreatic juice (lack of pancreatin) (Humbert et al., 2018) and bicarbonate (lower alkalinisation of the gastric content in the duodenum) (Robinson et al., 1990; Gelfond et al., 2013; Aburb et al., 2018), along with reduced bile salts secretion (Harries et al., 1979; Humbert et al., 2018) (**Figure 2B**). The resulting EPI-cystic fibrosis in vitro digestion model was first described by Asensio-Grau et al. (2018), and applied in the subsequent studies conducted by our group (Asensio-Grau, Peinado, Heredia & Andrés, 2018; Asensio-Grau, Calvo-Lerma, Heredia & Andrés, 2018; Paz-Yépez, Peinado, Heredia & Andrés 2018; Paz-Yépez, Peinado, Heredia & Andrés 2019; Asensio-Grau, Peinado, Heredia & Andrés, 2019).

The experimental design that was conducted in the study of lipolysis in all the target food groups had three objectives: 1) elucidating the role of the intestinal conditions (pH and bile salts concentration) on lipolysis, 2) finding out the optimal dose of enzymes

for each of the assessed foods (the dose that maximises lipolysis extent) and 3) describing the role of some food properties (mainly composition and matrix structure) on lipolysis extent. To achieve the first objective, the experimental design included the simulation of different combinations of intestinal conditions in order to mimic possible intestinal scenarios: worst-case (intestinal pH 6, bile salts concentration 1 mM), EPI-CF conditions with no biliary function alteration (pH 6, 10 mM), EPI-CF conditions with no bicarbonate secretion defect (pH 7, 1 mM) and healthy-like or normal conditions (pH 7, 10 mM). The doses of pancreatic enzyme supplements that were considered to be the range in which the optimal dose could fit was 1000, 2000, 3000 and 4000 lipase units per gram of fat (LU/g fat) (**Figure 2C**). This assumption was made on the basis of the current clinical guidelines for dosage and some preliminary experiments (Turk et al., 2016).

By means of this experimental approach, more than 1000 in vitro digestion experiments were conducted.

3. HOST-RELATED FACTORS IN PATIENTS WITH CYSTIC FIBROSIS

As above anticipated, dietary lipids' hydrolysis is in part conditioned by the medium in which the reaction occurs, i.e., the luminal gastrointestinal conditions. These are known as host-related factors, because they depend on the specific physiology of any individual. Normally, host-related factors are inherent to the health or disease condition of an individual, and cannot be modified unless by means of therapeutic treatments. In normal conditions, intestinal digestion occurs at pH 7, which is achieved after pancreatic bicarbonate has been secreted into the duodenum, the first part of the intestine (Gelfond et al., 2013). The alkaline pH of the medium results optimal for pancreatic lipase, which is one of the main enzymes contributing to total lipid digestion (Desnuelle & Savary, 1963).

The other key condition for optimal lipid digestion relates to bile salts secretion and concentration in the digestion medium (Sarkar, Ye & Singh, 2016). Healthy individuals secrete the necessary amount of biliary fluid to achieve a 10 mM concentration in the intestinal digestion medium (Minekus et al., 2014), which is above the critical micelle concentration. Bile salts have several roles in fat globules hydrolysis, besides the micelle formation capacity that is needed for further absorption (Hunter, 2001). First, they have the ability to displace proteins naturally adsorbed to the droplet surface, thus increasing the surface area available for lipases to adhere. Second, bile salts remove the products of lipolysis (free fatty acids) from the surface of the fat droplets, contributing to the prevention of product-inhibition of the enzymes (Maldonado-Valderrama, Wilde, Macierzanka & Makie, 2011).

Thus, both alkaline pH and high bile salts concentration are the main determinants of an optimal lipolysis in the intestine. In cystic fibrosis both are compromised, due to obstruction of the pancreatic duct (reduced or failed bicarbonate secretion) and the altered biliary function (reduced bile salts concentration up to ten times lower) (Robinson et al., 1990; Gelfond et al., 2013; Aburb et al., 2018; Harries et al., 1979 & Humbert et al., 2018). However, other gastric and intestinal digestion characteristics could have an effect on lipolysis, such as gastric pH, volume of secreted fluids, and bile salts composition in bile acids, among others.

According to the literature, altered gastrointestinal conditions could have an impact on lipid digestion. Thus, a specific study was conducted to integrally assess possible different combinations of gastrointestinal characteristics. Taking a nutritional supplement as a model food, combinations of gastric and intestinal pH, bile salts formulation and concentration, volume of simulated fluids secretion, and concentration of fat in the digestion medium were studied aiming at quantifying the effect of all these

parameters had on lipolysis extent (Calvo-Lerma, Fornés-Ferrer, Heredia & Andrés, 2019). As shown in **Figure 3**, intestinal pH was the most determinant parameter, as its relative effect accounted for 22.86% increase of lipolysis extent when the optimal pH is achieved (pH 7) compared to the reduced pH that is present in the EPI conditions. Following, but with a large lower effect, the fat concentration in the digestion medium increased 6.76% lipolysis extent comparing 0.7 with 4.2 g lipid/ml digestion fluids. The other factors showing a significant effect, although much lower than the others (<2 %) were bile salts concentration, gastric pH and formulation of bile salts with a high proportion of glycol-conjugated salts.

3.1. Intestinal pH and bile salts concentration affect differently lipolysis depending on the type of food

Indeed, the importance of intestinal pH and bile salts concentration has been repeatedly confirmed in the context of digestion of foods from different groups, when subjected to in vitro digestion (Calvo-Lerma et al., 2018). However, the effect is dependent of the type of food, some foods showing a greater influence either by the pH or the bile salts, both or none of them (**Figure 4**). The rationale behind this observation might be related to the complex digestion medium that results from the digestion of a food, depending on its characteristics, and even the cooking technique. For example, some protein digestion products might compete with bile salts for the adsorption onto the fat droplet surface in high protein foods, or foods with proteins with interfacial affinity (Wilde & Chu, 2011). However, in other foods this competitive phenomenon might not occur, leading to bile salts and its concentration governing the removal of lipolysis products from the fat droplet surface, thus determining the extent of lipolysis (Sakar, Horne & Singh, 2010). Another possible scenario would be related to colloidal properties

of the systems in which the removal effect of bile salts did not play such an important role in promoting the progress of lipolysis, thus letting the pH of the digestion fluids be the major determinant of the process, as lipase activity is highly dependent on this factor as well (Asensio-Grau, Peinado, Heredia & Andrés, 2018; Paz-Yépez, Peinado, Heredia & Andrés, 2019 a).

According to the results, four conclusions can be driven concerning host-related factors. First, when designing in vitro digestion models to assess lipolysis, amendments to the standard digestion conditions must be taken into account in order to adapt the environment to specific conditions such as EPI, as the results will be largely (intestinal pH) to moderately conditioned by these facts; this fact should be also considered in the context of other pathologies coursing with gastric, pancreatic or hepatic alterations, which are those needing the most research related to macronutrient digestion. Second, recommendations of dietary intake for patients subjected to PERT should be made, promoting the preference for those foods which lipolysis extents are not as much conditioned by the intestinal pH. Third, high fat diets should be advised to patients suffering from cystic fibrosis and EPI, as the higher the lipid concentration in the digestion medium, the highest enzyme efficacy is reached. And fourth, provided that intestinal pH is one of the most limiting factors of lipid digestion, in the treatment of EPI, clinical strategies to increase intestinal pH should be recommended, such as the use of proton pump inhibitors, which supress acidification of the gastric content, further facilitating alkaline medium during intestinal digestion.

2. DOSING CRITERIA FOR THE PANCREATIC ENZYME THERAPY

As one of the main objectives of the project, finding the optimal dose of the pancreatic enzyme supplement for the most relevant fat containing foods was pursued.

Despite current clinical guidelines recommend the dose should be within the range of 2000 to 4000 LU/g of dietary fat, lack of evidence is acknowledged (Turck et al., 2016). Thus, in all the in vitro digestion studies of different food products were conducted, 0, 1000, 2000, 3000 and 4000 LU/g fat were tested under the most unfavourable cystic fibrosis-EPI conditions.

As initially expected, lipolysis extent would be related with the enzyme dose/substrate ratio. Also, as expected, from a certain dose onwards, lipolysis would reach a maximum or asymptotic value. Indeed, as shown in **Figure 5**, in some foods, the asymptotic value for lipolysis extent was reached within the studied range of doses (A), while in some others, this asymptote was not observed. In addition, some foods could achieve the maximum of 100% lipolysis (either at high or low doses of enzymes, independently of the pattern observed), and some others could not.

These observations could enable the assignation of an optimal enzyme dose to every food, which was the dose allowing for maximum lipolysis. A specific data modelling approach was followed for such purpose, and revalidated in a pilot study (Calvo-Lerma, Fornés-Ferrer, Heredia & Andrés, 2019; Calvo-Lerma et al., 2019 c). On the other hand, this finding could evidence that lipolysis extent does not rely not only on the amount of fat in food, but also on many other factors, such as physicochemical properties of fats, organization of lipids in food products, lipid accessibility in food matrices and, interactions of non-lipid components of the matrix (Calvo-Lerma, Fornés-Ferrer, Heredia & Andrés, 2018). The following section provides further discussion.

3. INFLUENCE OF COMPOSITION AND FOOD MATRIX ON LIPID DIGESTION

There is a large number of parameters that can define the characteristics a food has in the context of lipid digestion. Foods are very complex materials integrated by nutrient and non-nutrient components such as carbohydrates, protein, fat, vitamins, minerals and fibre (Guo et al., 2017) . The food matrix is defined as the 3D structure that the interaction and assembly of macronutrients and other components confer to a food (Guo et al., 2017). It can be defined as the spatial architecture resulting from the assembly of macromolecules such as proteins, polysaccharides and lipids into a coordinated network (Ubbink, Burbidge & Mezzenga, 2008) . Most foods are complex, heterogeneous materials composed of structural elements or domains existing as solids, liquids and/or gases. The structure of all foods is provided by nature or imparted during processing and preparation. From this structure, some properties are derived, such as thermochemical or physicochemical, including texture and viscosity. The food matrix also plays a crucial role in how food interacts with the gastrointestinal tract and the resulting release and uptake of nutrients (Nyemb et al., 2014).

Given the well-known relevance of the food structure on the fate of lipid digestibility, Michalski et al. (2013) made an attempt to classify foods according to the lipid structure within the food matrix. This classification differentiated, for example, lipid droplets in the form of oil in water emulsion (like in milk and dairy products), intracellular lipid droplets and membrane structures (like in egg and meat) or lipid inclusion in carbohydrate or protein matrix (like in cheese or chocolate), among others. Taking this classification into account, a category was assigned to 52 foods belonging to a series including different groups, and the effect this category had on lipid digestion was evaluated (Calvo-Lerma, Fornés-Ferrer, Heredia and Andrés, 2018). In this study, it was evidenced that the type of lipid structure could indeed explain the difference in lipolysis extents obtained by different groups, both after normal intestinal digestion and especially

under EPI-CF simulated conditions. This study could also depict existing interactions between lipid with protein and lipid with carbohydrates, which can also explain the difference in lipolysis extent between different groups, also depending on the amount of fat present. Concretely, it was observed that foods with high content of protein or carbohydrate along with a low content of lipid, showed reduced lipolysis extent as compared to foods with high content of lipids.

Placing the focus on specific foods, and targeting food matrix and nutrient composition as the most relevant food related factors, the following sections summarise the main results obtained in egg (Asensio-Grau et al., 2018), cheese (Asensio-Grau et al., 2019), meat (Asensio-Grau et al., 2019), nuts (Paz-Yépez et al., 2018) and chocolate (Paz-Yépez et al., 2019).

3.1. Food matrix structure

For macronutrients digestion takes place, the food matrix has to be first disintegrated so the forming elements can be released to the digestion medium, where eventually digestive enzymes will carry out hydrolysis (Grundy, Carrière, Mackie & Gray, 2016). Particularly, lipid digestibility has showed to be closely related to the matrix degradation index in several food types (Fang, rioux, Labrie & Turgeon, 2016; Sarkar, Juan & Kolodziejczyk, 2015). There are several processes that impart the structure of a food matrix, besides the native structure a food may have *per se*. The spectrum of means by which the food matrix may be altered include the manufacturing or industrial processing, the cooking techniques that may be applied to make the food suitable to consume, and the mastication that some foods have to undertake to become swallowed.

An example of how the industrial processing changes a native matrix into different new structures concern cheese elaboration. Starting with the same cow milk, the ripening

time can deliver fresh, mild or aged cheese, and, despite the three of them result in similar nutrient composition, lipid digestion fate will be different, specifically because of the matrix effect (Asensio-Grau, Peinado, Heredia & Andrés, 2019).

A different effect the industrial processing can impart in foods relate to meat products. Meat can be consumed in its fresh form, with no alteration of the natural structure (beef steak, pork loin, chicken drumstick…), or after the mincing process most of the available products available in the supermarkets undertake (sausages, luncheon ham, hamburgers…). This disintegration of the meat pieces, implies the breakdown of the protein fibres that naturally entrap the fat particles, thus favours fat exposure to lipases in the digestion medium (Asensio-Grau, Calvo-Lerma, Heredia & Andrés, 2018).

As above-mentioned, the cooking technique indeed conditions the final structure a food will result in. In the case of egg, this has a great impact (Asensio-Grau, Peinado, Heredia & Andrés, 2018). For example, poached egg presents with a liquid yolk, where lipid molecules are naturally present. This liquid physical state makes the dilution of lipids in the digestive fluids relatively immediate. In contrast, hard egg, only with some extra time exposed to boiling water, acquires a solid yolk, and this implies more mechanical breakdown before lipid molecules can get diluted in the digestion fluids, and thus become available to lipases. Mixing the yolk, where lipids are, with the egg white, where proteins are present, results in a very complex structure that, when cooked in the form of an omelette, confers a matrix that is difficult to disintegrate and in consequence lipid digestibility is hindered.

The effect of the food matrix is also present when it comes to mastication. In a study conducted with nuts (peanut and walnut) the impact of particle size resulting after mastication simulation showed to be determinant in the extent of lipolysis (Paz-Yépez, Peinado, Hereida & Andrés, 2019). In fact, the degree of disintegration at the oral stage

was the most effective variable, even more than the intestinal conditions or the type of lipid contained in the two different nuts, on lipolysis extent.

Overall, **Figure 6** presents an overview of the close relationship food matrix disintegration and lipolysis extent present in some foods from different natures.

3.2. Food composition

Once the effect of the food matrix structure is confirmed, the following step should be exploring what happens in those foods with the same food matrix structure, but different nutritional composition. To address this research question a study was conducted on the basis of white, milk and dark chocolate bars (Paz-Yépez, Peinado, Heredia & Andrés et al., 2019). Despite these three matrices have in common the compounds inherent to cocoa beans, different parts are used in the manufacturing of the resulting chocolates, and different additives are included to the final product too. In this sense, the lowest lipolysis extent was found in dark chocolate, this result being attributed to the presence of polyphenols belonging to the cocoa paste that is present in higher amounts than in the other two varieties of chocolate. polyphenols, have shown to have an inhibitory effect of lipases activity (Cha, Song, Kim, & Pan, 2012).. Milk chocolate, conversely, showed the highest lipolysis extent, possibly because of the high proportion of milk fat, which is presented in an easily accessible form to lipases, plus has shown to be smoothly hydrolysed by pancreatic lipases. Finally, white chocolate resulted in the lowest lipolysis, possibly because it is mainly made of cocoa butter, in fat may present in a bulky form during digestion, thus preventing the accessibility of lipases.

Considering all the presented results concerning food properties and lipolysis together, some consistent remarks can be made: 1) the type and structure of food matrix is to be considered when assessing lipolysis, as the breakdown of the matrix will certainly

determine the release of fat globules to the digestion medium, where lipolysis shall eventually occur. Therefore, the processes taking place prior digestion, such as industrial manufacturing, cooking and mastication, can be tailored to modulate desired lipolysis extent. In addition to the food matrix structure, the presence of nutrients and the interactions occurring among them can further determine lipid digestibility.

4. FROM LAB TO DAILY LIFE IN PATIENTS WITH CYSTIC FIBROSIS

The knowledge generated throughout the in vitro digestion studies was translated into a direct practical application in the treatment of PERT. The results from the lipid digestibility studies in 52 foods, covering the range of the fat-containing foods conforming the sources of daily lipid intake in patients with cystic fibrosis, were modelled with the aim of obtaining a theoretical optimal dose (TOD) for each of the foods. The TOD is the dose that allows for maximum lipolysis under the altered intestinal conditions occurring in EPI-cystic fibrosis. The TOD each single food was assigned with was also extrapolated to other similar foods, overall conforming a database. For example, the TOD predicted for salmon was also assigned to other fatty fish, such as mackerel or sardine; and the TOD for a regular biscuit was extrapolated to other varieties of regular biscuit. The adequacy of the TOD as a means of optimally adjusting the dose of enzymes was previously validated in a pilot study, in which participating patients obtained median 90% coefficient of fat absorption when applying the TOD (Calvo-Lerma et al., 2019).

The database containing the TOD values for fat-containing foods entails high potential of applicability in supporting patients in need of pancreatic enzyme supplements when it comes to dose adjustment. The most suitable approach to bring it available and practical to the patients was to integrate it in a mobile app, by means of which patients could report the foods they eat and the system tells the optimal dose of enzymes in real

time. This approach was previously described by our group (Calvo-Lerma et al., 2017) and the result of the present study implies the successful achievement of the cornerstone to make the system viable: the TOD database.

The described system was successfully implemented into MyCyFAPP mobile app (Floch et al., 2018). Thereafter, the self-management app was tested for effectiveness in a European multinational clinical trial. During 6 months, 170 paediatric patients with cystic fibrosis and pancreatic insufficiency used the app to adjust the dose of pancreatic enzyme supplements. The results showed improved quality of life, reduced gastrointestinal symptoms and improved nutritional status (Boon et al., 2019). This way, the milestone of self-management of dose of pancreatic enzyme supplements by means of a multidisciplinary-driven evidence-based method was reached, covering one of the priorities of Horizon 2020 Research and Innovation programme of the European Union: Self management of health and disease; citizen engagement and mHealth.

However, despite of positive results achieved in the clinical practice, further research in studying lipid digestion in the framework of EPI should be conducted, as only a first step has been taken towards characterising lipolysis, and other more complex approaches, such as studying co-digestion of different foods (meal factors), could offer a more accurate and realistic solution.

5. CONCLUSIONS

Summing up, the following remarks can be considered as the lessons learnt throughout the development of MyCyFAPP project:

1. Static methods are limited but have a potential for massive screening assessing luminal digestion process

2. Knowledge regarding lipolysis in real foods has been generated, including the identification of existing interactions among macronutrients and the importance of the food matrix structure; however, the field is still unexplored for lipolysis characterisation when foods are co-digested and meal factors occur.

3. Gastrointestinal conditions in pancreatic insufficiency affect lipid digestion differently depending on food characteristics. These findings could be extrapolated to other diseases related to EPI. Also, the approach followed in this project could support the generation of new tools for self-management of chronic diseases related to maldigestion.

4. Lipolysis characterisation in the study foods could be used for establishing dietary recommendations in patients with cystic fibrosis and EPI, by promoting the intake of those foods more easily achieving high lipolysis extents.

5. In vitro digestion models can truly address health problems and provide the necessary evidence to develop new therapies

Knowledge from food research area has approached the medical field with the aim of bringing new findings related to food digestion into a robust and evidence-based method to optimise pancreatic enzyme replacement therapy, a backbone in the nutritional therapy of cystic fibrosis, which up to date was implemented with no other guidance than the empiric procedure. Therefore, a synergy between basic and clinical research has made possible a successful translation of food science results to clinical practice having food digestion as a common focus in both.

The conclusion of the present work is that current knowledge about food systems and their properties must be taken into account when clinically recommending a dose of pancreatic enzyme supplements, and indeed, a newly developed tool including a recommended optimal dose for every food has been made available: the TOD database.

The positive results arising from the first prospective and multicentre validation of the system guarantee the implementation of a scientifically valid method to adjust pancreatic enzyme replacement therapy in the near future as part of a routine clinical treatment.

In addition, two final remarks summarise the work performed: 1) The first evidence-based method to adjust PERT was established; and 2) New knowledge was generated in the field of food and lipid digestion.

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6. REFERENCES

Aburub, A., Fischer, M., Camilleri, M., Semler, J. R., & Fadda, H. M. (2018). Comparison of pH and motility of the small intestine of healthy subjects and patients with symptomatic constipation using the wireless motility capsule. International Journal of Pharmaceutics, 544, 158–164.

Asensio-Grau, A., Peinado, I., Heredia, A., & Andrés, A. (2018). Effect of cooking methods and intestinal conditions on lipolysis, proteolysis and xanthophylls bioaccessibility of eggs. Journal of functional foods, 46, 579-586.

Asensio-Grau, A., Calvo-Lerma, J., Heredia, A., & Andrés, A. (2019). Fat digestibility in meat products: influence of food structure and gastrointestinal conditions. International journal of food sciences and nutrition, 70, 530-539.

Asensio-Grau, A., Peinado, I., Heredia, A., & Andres, A. (2019). In vitro study of cheese digestion: Effect of type of cheese and intestinal conditions on macronutrients digestibility. LWT, 108278.

Boon, M., Calvo-Lerma, J., Claes, I., Havermans, T., Fornes, V., Asseiceira, I., ... & Barreto, C. (2019). ePS5. 08 MyCyFAPP project: use of a mobile application for self-management of PERT improves gastrointestinal related quality of life in children with cystic fibrosis. Journal of Cystic Fibrosis, 18, S52-S53.

Calvo-Lerma, J., Martinez-Jimenez, C. P., Lázaro-Ramos, J. P., Andrés, A., Crespo-Escobar, P., Stav, E., ... & Ribes-Koninckx, C. (2017). Innovative approach for self-management and social welfare of children with cystic fibrosis in Europe: development, validation and implementation of an mHealth tool (MyCyFAPP). BMJ open, 7(3), e014931.

Calvo-Lerma, J., Fornés-Ferrer, V., Asensio-Grau, A., Paz-Yépez, C., Heredia, A., Ribes-Koninckx, C., & Andrés, A. (2019). P284 Food characteristics as determinants of PERT requirements: summary of the in vitro digestion experiments of MyCyFAPP project. Journal of Cystic Fibrosis, 18, S137.

Calvo-Lerma, J., Hulst, J. M., Asseiceira, I., Claes, I., Garriga, M., Colombo, C., ... & Ribes-Koninckx, C. (2017). Nutritional status, nutrient intake and use of enzyme supplements in paediatric patients with Cystic Fibrosis; a European multicentre study with reference to current guidelines. Journal of Cystic Fibrosis, 16(4), 510-518.

Calvo-Lerma, J., Fornés-Ferrer, V., Peinado, I., Heredia, A., Ribes-Koninckx, C., & Andrés, A. (2019). A first approach for an evidence-based in vitro digestion method to adjust pancreatic enzyme replacement therapy in cystic fibrosis. PloS One, 14, e0212459.

Calvo-Lerma, J., Fornés-Ferrer, V., Heredia, A., & Andrés, A. (2019). In vitro digestion models to assess lipolysis: The impact of the simulated conditions for gastrointestinal pH, bile salts and digestion fluids. Food Research International, 108511.

Calvo-Lerma, J., Hulst, J., Boon, M., Martins, T., Ruperto, M., Colombo, C., ... & Ribes-Koninckx, C. (2019). The relative contribution of food groups to macronutrient intake in children with cystic fibrosis: A European multicenter assessment. Journal of the Academy of Nutrition and Dietetics, 119, 1305-1319.

Calvo-Lerma, J., Fornés-Ferrer, V., Heredia, A., & Andrés, A. (2018). In Vitro Digestion of Lipids in Real Foods: Influence of Lipid Organization Within the Food Matrix and Interactions with Nonlipid Components. Journal of Food Science, 83(10), 2629-2637.

Calvo-Lerma, J., Hulst, J., Boon, M., Colombo, C., Masip, E., Ruperto, M., ... & Roca, M. (2019). Clinical validation of an evidence-based method to adjust Pancreatic Enzyme Replacement Therapy through a prospective interventional study in paediatric patients with Cystic Fibrosis. PloS One, 14, e0213216.

Cha, K. H., Song, D. G., Kim, S. M., & Pan, C. H. (2012). Inhibition of gastrointestinal lipolysis by green tea, coffee, and gomchui (Ligularia fischeri) tea polyphenols during simulated digestion. Journal of agricultural and food chemistry, 60, 7152-7157.

Desnuelle, P., & Savary, P. (1963). Specificities of lipases. Journal of Lipid Research, 4, 369–384.

Elborn, J.S. (2016). Cystic fibrosis. The Lancet, 388, 2519-2531.

Fang, X., Rioux, L. E., Labrie, S., & Turgeon, S. L. (2016). Disintegration and nutrients release from cheese with different textural properties during in vitro digestion. Food Research International, 88, 276-283.

Floch, J., Zettl, A., Fricke, L., Weisser, T., Grut, L., Vilarinho, T., ... & Schauber, C. (2018). User needs in the development of a health app ecosystem for self-management of cystic fibrosis: user-centered development approach. JMIR mHealth and uHealth, 6, e113

Gelfond, D., Ma, C., Semler, J., & Borowitz, D. (2013). Intestinal pH and gastrointestinal transit profiles in cystic fibrosis patients measured by wireless motility capsule. Digestive Diseases and Sciences, 58, 2275–2281.

Guo, Q., Ye, A., Bellissimo, N., Singh, H., & Rousseau, D. (2017). Modulating fat digestion through food structure design. Progress in Lipid Research, 68, 109-118.

Grundy, M. M., Carrière, F., Mackie, A. R., Gray, D. A., Butterworth, P. J., & Ellis, P. R. (2016). The role of plant cell wall encapsulation and porosity in regulating lipolysis during the digestion of almond seeds. Food & Function, 7, 69-78.

Harries, J. T., Muller, D. P., McCollum, J. P., Lipson, A., Roma, E., & Norman, A. P. (1979). Intestinal bile salts in cystic fibrosis: Studies in the patient and experimental animal. Archives of Disease in Childhood, 54, 19–24.

Humbert, L., Rainteau, D., Tuvignon, N., Wolf, C., Seksik, P., Laugier, R., & Carrière, F. (2018). Postprandial bile acid levels in intestine and plasma reveal altered biliary circulation in chronic pancreatitis patients. Journal of Lipid Research, 59, 2202–2213.

Hunter, J. E. (2001). Studies on effects of dietary fatty acids as related to their position on triglycerides. Lipids, 36, 655-668.

Maldonado-Valderrama, J., Wilde, P., Macierzanka, A., & Mackie, A. (2011). The role of bile salts in digestion. Advances in colloid and interface science, 165, 36-46.

Ménard, O., Cattenoz, T., Guillemin, H., Souchon, I., Deglaire, A., Dupont, D., & Picque, D. (2014). Validation of a new in vitro dynamic system to simulate infant digestion. Food chemistry, 145, 1039-1045..

Michalski, M. C., Genot, C., Gayet, C., Lopez, C., Fine, F., Joffre, F., ... & steering committee of RMT LISTRAL. (2013). Multiscale structures of lipids in foods as parameters affecting fatty acid bioavailability and lipid metabolism. Progress in lipid research, 52, 354-373.

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T. O. R. S. T. E. N., Bourlieu, C., ... & Dufour, C. (2014). A standardised static in vitro digestion method suitable for food–an international consensus. Food & function, 5, 1113-1124.

Nyemb, K., Guérin-Dubiard, C., Dupont, D., Jardin, J., Rutherfurd, S. M., & Nau, F. (2014). The extent of ovalbumin in vitro digestion and the nature of generated peptides are modulated by the morphology of protein aggregates. Food chemistry, 157, 429-438.

Paz-Yépez, C., Peinado, I., Heredia, A., & Andrés, A. (2019). Influence of particle size and intestinal conditions on in vitro lipid and protein digestibility of walnuts and peanuts. Food Research International, 119, 951-959

Paz-Yépez, C., Peinado, I., Heredia, A., & Andrés, A. (2019). Lipids digestibility and polyphenols release under in vitro digestion of dark, milk and white chocolate. Journal of functional foods, 52, 196-203.

Robinson, P. J., Smith, A. L., & Sly, P. D. (1990). Duodenal pH in cystic fibrosis and its relationship to fat malabsorption. Digestive Diseases and Sciences, 35, 1299-1304.

Sarkar, A., Ye, A., & Singh, H. (2016). On the role of bile salts in the digestion of emulsified lipids. Food Hydrocolloids, 60, 77–84.

Sarkar, A., Horne, D. S., & Singh, H. (2010). Interactions of milk protein-stabilized oil-in-water emulsions with bile salts in a simulated upper intestinal model. Food Hydrocolloids, 24, 142–151.

Sarkar, A., Juan, J. M., Kolodziejczyk, E., Acquistapace, S., Donato-Capel, L., & Wooster, T. J. (2015). Impact of protein gel porosity on the digestion of lipid emulsions. Journal of agricultural and food chemistry, 63, 8829-8837.

Smith, C., Baveja, R., Grieb, T., & Mashour, G. A. (2017). Toward a science of translational science. Journal of clinical and translational science, 1, 253-255.

Surkis, A., Hogle, J. A., DiazGranados, D., Hunt, J. D., Mazmanian, P. E., Connors, E., ... & Aphinyanaphongs, Y. (2016). Classifying publications from the clinical and translational science award program along the translational research spectrum: a machine learning approach. Journal of translational medicine, 14, 235.

Turck, D., Braegger, C. P., Colombo, C., Declercq, D., Morton, A., Pancheva, R., ... & Schneider, S. M. (2016). ESPEN-ESPGHAN-ECFS guidelines on nutrition care for infants, children, and adults with cystic fibrosis. Clinical Nutrition, 35, 557-577.

Ubbink, J., Burbidge, A., & Mezzenga, R. (2008). Food structure and functionality: a soft matter perspective. Soft matter, 4, 1569-1581.

Wilde, P. J., & Chu, B. S. (2011). Interfacial & colloidal aspects of lipid digestion. Advances in Colloid and Interface Science, 165, 14-22.

Woestenenk, J. W., van der Ent, C. K., & Houwen, R. H. (2015). Pancreatic enzyme replacement therapy and coefficient of fat absorption in children and adolescents with cystic fibrosis. Journal of pediatric gastroenterology and nutrition, 61, 355-360.

Wouthuyzen-Bakker, M., Bodewes, F. A., & Verkade, H. J. (2011). Persistent fat malabsorption in cystic fibrosis; lessons from patients and mice. Journal of Cystic Fibrosis, 10, 150-158

FIGURE LEGENDS

Figure 1. Overview of the three main factors conditioning lipid digestion in cystic fibrosis and exocrine pancreatic insufficiency: host related factors, food intake, and dose of pancreatic enzyme supplement

Figure 2. Overview of the static in vitro digestion experimental framework conducted to explore lipid digestibility of the foods contributing to dietary lipid intake (A), simulating the intestinal conditions of healthy subjects and patients with cystic fibrosis (CF) and exocrine pancreatic insufficiency (EPI) (B) and using different doses of the pancreatic enzyme supplements used in pancreatic enzyme replacement therapy (C).

Figure 3. The relative effect of gastric and intestinal conditions on lipolysis extent (%), expressed as the odds ratio (OR) obtained by means of linear mixed regression models, considering the food as a random effect.

Figure 4. Variable effect of the intestinal medium pH and bile salts concentration on lipolysis extent depending on the type of food.

Figure 5. Two possible tendencies observed regarding lipolysis extent achieved as function of pancreatic enzyme supplement dose: a) increasing up to a certain dose after which lipolysis is maintained or decreased; b) continuously increasing with the dose.

Figure 6. Non-linear correlation between matrix degradation index during digestion in a sample of egg, cheese and meat products and the lipolysis extent achieved after intestinal digestion.