Clinical evaluation of an evidence-based method based on food characteristics to adjust pancreatic enzyme supplements dose in Cystic Fibrosis

Joaquim Calvo-Lerma[1,2*], Mieke Boon[3], Carla Colombo[4], Barbara de Koning[5], Inês Asseiceira[6], Maria Garriga[7], Maria Roca[1], Ine Claes[3], Anna Bulfamante[4], Sylvia Walet[5], Luisa Pereira[6], Mar Ruperto[7], Etna Masip[1], Andrea Asensio-Grau[2], Arianna Giana[4], Philine Affourtit[5], Ana Heredia[2], Saioa Vicente[7], Ana Andrés[2], Kris de Boeck[3], Jessie Hulst[5,8·], and Carmen Ribes-Koninckx[1]

1 Instituto de Investigación Sanitaria La Fe de Valencia. Cystic Fibrosis Unit. 46026 Valencia, Spain.

2 Universitat Politècnica de València, Research Institute of Food Engineering for Development. 46022 Valencia, Spain.

3 Department of pediatrics, Center for Cystic Fibrosis, University Hospital Leuven. Leuven, Belgium

4 CF Center, Università degli Studi di Milano. Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico. 20122 Milan, Italy

5 Department of Pediatrics, div of Gastro-Enterology and div of Respiratory Medicine and Allergology, Erasmus MC- Sophia Children's Hospital, University Hospital Rotterdam. Rotterdam, Netherlands.

6 Centro de Fibrose Quística, Hospital de Santa Maria, Lisboa. Lisbon, Portugal

7 Hospital Universitario Ramón y Cajal. Cystic Fibrosis Unit. 28034 Madrid, Spain.

8 Division of Gastroenterology, Hepatology and Nutrition, The Hospital for Sick Children, Toronto, Canada

* Corresponding author; joacalle@upv.es; +34 961246712; Unviersitat Politècnica de València Camino de Vera s/n. 46022 València (Spain)

ABSTRACT

Background: Patients with cystic fibrosis (CF) and pancreatic insufficiency need pancreatic enzyme replacement therapy (PERT) for dietary lipids digestion. There is limited evidence for recommending the adequate PERT dose for every meal, and controlling steatorrhea remains a challenge. This study aimed to evaluate a new PERT dosing method supported by a self-management mobile-app.

Methods: Children with CF recruited from 6 European centres were instructed to use the app, including an algorithm for optimal PERT dosing based on *in vitro* digestion studies for every type of food. At baseline, a 24h self-selected diet was registered in the app, and usual PERT doses were taken by the patient. After 1 month, the same diet was followed, but PERT doses were indicated by the app. Change in faecal fat and coefficient of fat absorption (CFA) were determined.

Results: 58 patients (median age 8.1 years) participated. Baseline fat absorption was high: median CFA 96.9%, median 2.4g faecal fat). After intervention CFA did not significantly change, but range of PERT doses was reduced: interquartile ranges narrowing from 1447-3070 at baseline to 1783-2495 LU/g fat when using the app. Patients with a low baseline fat absorption (CFA<90%, n=12) experienced significant improvement in CFA after adhering to the recommended PERT dose (from 86.3 to 94.0%, p=0.031).

Conclusion: the use of a novel evidence-based PERT dosing method, based on in vitro fat digestion studies incorporating food characteristics, was effective in increasing CFA in patients with poor baseline fat absorption and could safely be implemented in clinical practice.

Keywords: cystic fibrosis, pancreatic insufficiency, digestion, paediatrics, in vitro digestion, pancreatic enzyme replacement therapy, coefficient of fat absorption.

1. INTRODUCTION

Infection and inflammation cycles in the lungs are the major cause of poor prognosis in Cystic Fibrosis (CF) [1]. However, the obstruction of the pancreatic duct governs the other area of the pathology needing treatment: exocrine pancreatic insufficiency,-which affects 85% of patients [2].

The secretion of digestive enzymes in the intestine is impaired and hydrolysis of dietary lipids is compromised the most, as pancreatic lipase is the main agent for lipid digestion [3]. Maldigestion of lipids seriously compromises nutrient absorption, and consequently fat and lipid-soluble vitamins are excreted with faeces causing poor weight gain. In addition, maldigestion also causes gastrointestinal complaints such as diarrhoea, bloating, and abdominal pain, which can affect the quality of life [4]. The long-term consequences imply a decline in the nutritional status, which is strongly associated with disease prognosis and survival [5,6].

Reverting this situation is possible by means of pancreatic enzyme replacement therapy (PERT), which consists of the exogenous administration of encapsulated pancreatic enzymes with every meal [7]. The current guidelines on nutrition in CF, however, can only make a “low-evidence” recommendation for the optimal dosing of PERT supplements [8], as no study has been able to establish an association between dose and fat absorption [3]. In fact, up to date there are a few strategies to manage patients with persistent steatorrhea, which is a recurrent presence of faecal fat along with gastrointestinal discomfort, overall hindering nutritional status [9].

In this context of limited evidence about adequate PERT dosing, recommending fat-rich diets from healthy foods to guarantee high energy intake, is the mainstream recommendation to support the nutritional status [10,11]. However, in the past few years, it has been recognized that food characteristics are important determinants of PERT

efficacy, suggesting including them as a plausible criterion in order to establish optimal doses. The rationale behind relies on the food matrix structure, whose disintegration during digestion, determines fat release and accessibility of digestive enzymes [12-15].

In previous research, an in vitro digestion model was set up to study fat digestion in foods when using different doses of PERT supplements. The model simulated the gastrointestinal conditions that are present in CF [16-20], and foods belonging to different groups (dairy, cheese, meat, fish, eggs, nuts, bakery, pastries, oils, butter, chocolate, and other products) were assessed [13, 21-25]. Modelling of the results allowed for determining the optimal dose for each food (i.e., the TOD) [30], either 1000, 2000, 3000 or 4000 LU/g of fat. The assigned TOD was not solely related to the amount of fat in food, but was also dependent on food matrix structure and interactions among other macronutrients (protein and carbohydrate) with lipids. In this sense, foods with high protein or carbohydrate content and low content of fat, resulted in lower lipolysis than those with high fat and either low carbohydrate or protein content. In terms of food groups, those presenting an oil-in-water emulsion structure, such as milk and dairy (e.g., whole cow milk required 2000 LU/g fat, and yoghurt 1000 LU/g fat), were more easily digested, thus required lower doses, than others with more complex structures such as foods presenting lipid inclusion in protein matrices or tissues, as in the case of fish or nuts (examples of required doses being 4000 LU/g fat for salmon and peanuts) [13].

A pilot study, applying this ~~model~~ method to adjust PERT dose showed a good median coefficient of fat absorption (CFA, 90%) in a cohort of 42 children [16]. Another qualitative study by our consortium concluded that patients with CF would experience potential benefits from adopting a self-management app for PERT dose advice [17]. So, the tool was implemented into a mobile app for patients' self-management and evaluated

in a 6-month multicentre clinical trial, the main outcome being the improvement in gastrointestinal-related quality of life [18].

The present sub-study aimed at assessing the novel PERT dosing method, incorporating food characteristics, and supported by a mobile app, compared to the regular dosing criteria, by means of evaluating the coefficient of fat absorption.

2. METHODS

2.1. Subjects and study design

The present study was conducted as a pre-planned sub-study within MyCyFAPP Clinical Trial [29] (**Figure 1**), a multicentre study in children with CF and pancreatic insufficiency from the centres in Lisbon, Madrid, Valencia, Milan, Leuven and Rotterdam. At the run-in visit, patients were given a written study protocol along with a smartphone device containing the "MyCyFAPP" self-management app. Patients were asked to participate in the present sub-study at that time as well. The clinical trial consisted of a run-in visit and three subsequent visits at month 1, 3 and 6; this sub-study was conducted from run-in visit (visit 0) through the visit at month 1 (visit 1).

After patients' inclusion in the sub-study, 24h food records had to be completed through the "food recording" menu in the app, along with the accompanying doses of PERT according to patients' previous usual experience (visit 0). The "optimal PERT dose" feature was disabled while performing the first food recording. Subsequently (within 2 weeks from inclusion in the study), healthcare teams remotely enabled the "optimal PERT dose" functionality, with patients being notified. At visit 1, patients were asked to repeat the same 24h diet as one month prior and record it, this time accompanied with the optimal dose of PERT being suggested in real time by the app calculation algorithm. Patients were asked to indicate whether the dose taken differed from the dose

recommended by the app, although strong emphasis was made to adhere to the prescribed dose. At both study visits, patients were asked to collect faecal samples, according to a strict protocol to enable accurate calculation of CFA [30]. In short, the study protocol consisted of the following:

- Visit 0: self-selected diet during 24h, free PERT dose, accompanying faecal collection
- Visit 1: self-selected diet during 24h, app PERT dose, accompanying faecal collection

Inclusion criteria consisted of confirmed diagnosis of CF by a sweat chloride ≥ 60 mEq/L and/or the presence of 2 disease-causing mutations in the CFTR gene, confirmed diagnosis of exocrine pancreatic insufficiency by faecal elastase values (FE1) < 200 mcg/g of faeces, treatment with PERT and age between one and 18 years. On the other hand, patients with acute infections or acute abdominal pain, and patients who recently (<3 months) started CFTR modulation therapy were excluded [29].

The study protocol was approved by the Ethical Committees of all the participating centres. The clinical trial was registered in the Spanish Agency of Drugs and Medical Devices (Ministry of Health), with reference number 645/17/EC. The trial was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki).

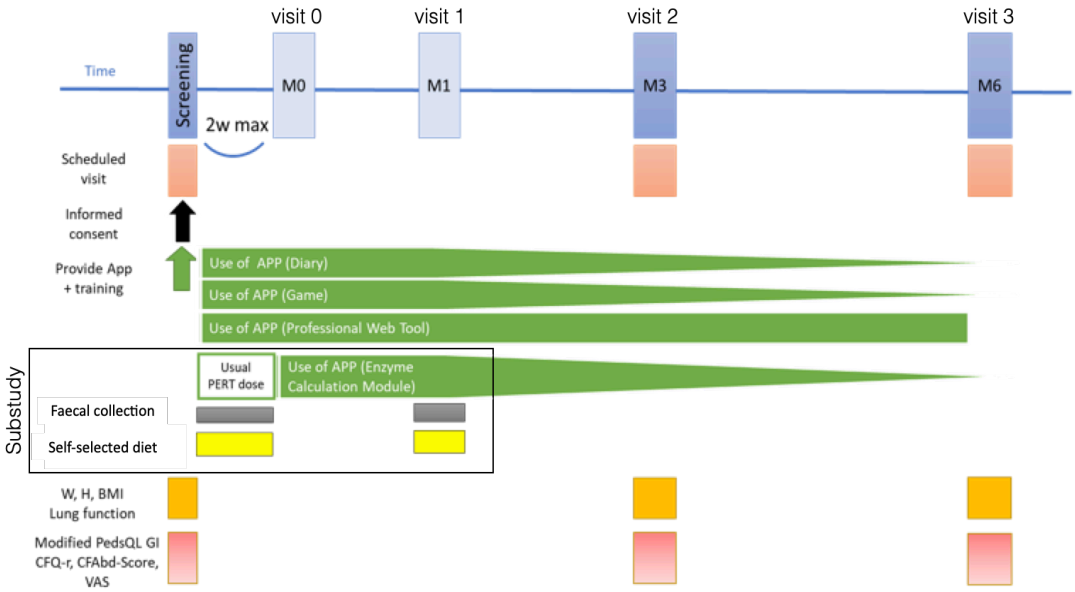

Figure 1. Schematic overview of the study design of the whole clinical trial [29]. The substudy included within included faecal collection and the accompaining 24h diet record along with PERT dose at both visit in month 0 and visit in month 1.

2.2. Interventional tool: app-supported optimal enzyme dose prediction

The core part of the self-management app was the "optimal enzyme dose prediction". It was run by the sequence of different components (**Figure 2**). The main element was a database including a "theoretical optimal dose" of enzyme supplements (TOD) and the content of fat (g fat/100 g of food) for all food items (A). The TOD was expressed as lipase units (LU) per gram of dietary fat. The TOD database ran in the background of the "food recording" feature so that when consumed food items and quantity in a meal were reported by the patient (B), a summation of the individual TOD for the consumed amount of each food item was done (C). Once a whole meal was registered, the app displayed the 'real time predicted optimal amount of enzymes for that specific meal in terms of LU, along with the dose expressed as number of PERT capsules needed (D). In the settings of the app, patients indicated the strength of the capsules they usually took, so that the app could display the possible combinations of number of

capsules recommended for each meal. Then, patients had to indicate the dose actually taken for each specific meal. The system was protected with Intellectual Property Rights in the Spanish Registry Office (Reference: O00012831e1800029158). All the data registered in the app were synchronised with the clinical trial server.

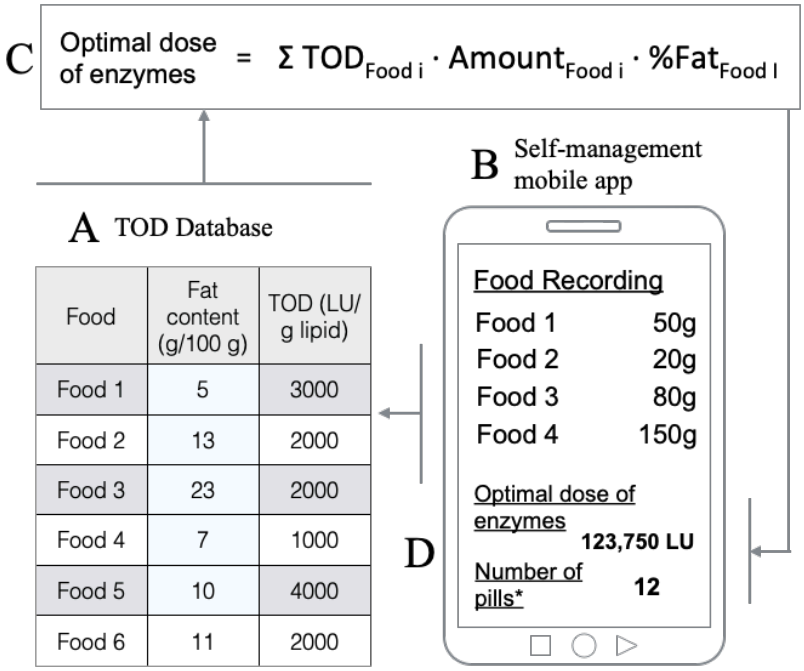

Figure 2. Schematic overview of the components supporting the prediction of the optimal dose of enzyme supplements. The core item is the Theoretical optimal dose database, in which all foods regularly consumed are assigned with a TOD value in LU/g fat * The translation into "number of capsules", given the recommended dose in LU, is made considering the lipase content of each capsule, either 5,000, 10,000 or 25,000 LU, which is pre-set in the settings of the app.

2.3. Study data collection

Biometry and lung function. At visit 1, patient characteristics (age, biometry and lung function) were measured and entered in an online electronic Case Report Form (eCRF). From these data, weight z-score, height z-score, BMI z-score (expressed according to CDC references [31]), and lung function (FEV1, [32]) were calculated.

Data collected through the app. Food records registered through the app were processed to calculate energy and macronutrient intake. From the optimal enzyme dose

prediction module, both recommended dose by the app, and actual dose reported by the patients were retrieved in terms of LU. With these data, the dose was expressed as LU/g of dietary fat.

Faecal collection: For accurate faecal collection, encapsulated dyes (E-120, red carmine and E-132, indigo blue) were ingested at start and end of the 24h food recording to identify the faeces specifically pertaining to the study period [30]. Then, collections were frozen (-20 to -80 ºC) and shipped to the central lab in Instituto de Investigación Sanitaria La Fe (Valencia, Spain) for analysis. Faeces were mixed with 750 W stirrers (Braun MQ735) until complete homogenisation (approx. 5 minutes per sample, depending on the consistency and volume). Total faecal fat was quantified on homogenised faecal samples (10 g) with infrared spectroscopy, the gold standard to analyse fat in faeces [8]. The CFA was calculated as the percentage of grams of fat excreted in the collected faeces relative to the grams of fat in the test diet.

2.4. Statistical analysis

The sample size calculation for the whole clinical trial was performed on the basis of improvement in the primary outcome, i.e., quality of life [30]. For the present study a specific sample size calculation was not performed in order not to restrict the inclusion of patients and because of the exploratory nature of the substudy goal.

All the obtained data were summarised as mean and standard deviation or median and 1st and 3rd quartiles. Patients with missing information regarding doses of PERT and/or food records were identified and excluded from analysis. Patients with fat malabsorption (CFA <90%) at the run-in visit were considered separately. A linear mixed regression model was applied to determine significant changes in CFA and faecal fat between the two study visits, in the whole cohort and in the subgroup of patients with fat

malabsorption. Statistical parameters were expressed with the p-value (considered significant when <0.05) along with the 95% confidence interval (CI) of the estimated effect.

3. RESULTS

3.1. Patients characteristics

Eighty-four subjects were recruited for this study, but only 58 patients, aged between 2.2 and 17.4 years old, were included for analysis according to the data accuracy criteria, which was having registered both food intake and PERT dose in the app, at both time points. No drop-outs were registered ~~along~~ during the 1-month duration. All of the patients were pancreatic insufficient as confirmed by FE1 < 200 μg/g faeces, and none of them had previous GI surgery or had short bowel syndrome. Gender distribution was equal, the median age of the cohort was 8.1 (5.2, 10.2) years old and forced expired volume in one second (FEV_1) was -0.42 (-1.13, 0.26) z-score. The use of proton pump inhibitors was registered in 16 subjects. Most of the patients (n = 54) presented a Class I or II mutation in one allele, being F508del the most frequent (n = 52) and in homozygosis in 33 patients. Forty-one patients had Class I or II mutation in both alleles. Biometry per centre data are presented in **Table 1**.

Table 1. Demographic characteristics of the study cohort (n= 58) expressed as median (1st and 3rd Q) or number.

	Lisbon (n = 4)	Madrid (n = 6)	Valencia (n = 8)	Milan (n = 6)	Leuven (n = 26)	Rotterdam (n = 8)	Total (n = 58)
Gender (n male)	3	4	4	3	13	2	**29**
Age (years)	6.4	8.3	8.2	9.3	8.5	4.4	**8.1**

	(5.2, 7.6)	(7.8, 10.1)	(6.5, 11.4)	(9.0, 11.0)	(5.4, 11.3)	(2.9, 6.3)	**(5.2, 10.2)**
Weight (z-score)	-0.01	-0.51	-0.37	-0.08	-0.46	0.27	**-0.36**
	(-0.18,	(-1.38, -	(-0.04,	(-0.92,	(-0.97,	(-0.13,	**(-0.74,**
	0.13)	0.44)	0.23)	0.60)	0.07)	0.69)	**0.16)**
Height (z-score)	-0.43	-1.10	-0.22	-0.44	-0.60	0.21	**-0.51**
	(-0.58,	(-1.13, -	(-0.44,	(1.05,	(-0.82,	(0.02,	**(-0.85,**
	0.05)	0.75)	0.34)	0.12)	0.48)	0.59)	**0.47)**
BMI (z-score)	0.03	-0.06	-0.10	-0.32	-0.53	0.33	**-0.2**
	(-0.11,	(-1.36,	(-0.26,	(-1.35,	(-0.92,	(-0.20,	**(-0.91,**
	0.19)	0.18)	0.19)	0.89)	0.34)	0.60)	**0.38)**

3.2. Change in PERT dose

A small (100 units) and non-significant decrease in mean PERT dose was seen at visit 1 compared to visit 0, median values being 2228 and 2338 LU/g fat, respectively. However, the interquartile range at visit 1 was significantly reduced from 1447 - 3070 LU/g fat at visit 0 to 1783-2495 LU/g fat at visit 1 ($p<0.01$) (**Figure 3**A), and it is best appreciated when looking at the lipase unit distribution per centre (**Figure 3**B). In Lisbon and Valencia PERT doses increased at visit 1, while in Milan, Leuven and Rotterdam, the dose tended to decrease; all doses in all centres, thus, coming closer to the doses recommended by the app (from 1000 to 4000 LU/g fat). In particular, in Leuven and Rotterdam, the centres with the widest ranges in PERT doses at visit 0, a large decrease in range when using the app was observed.

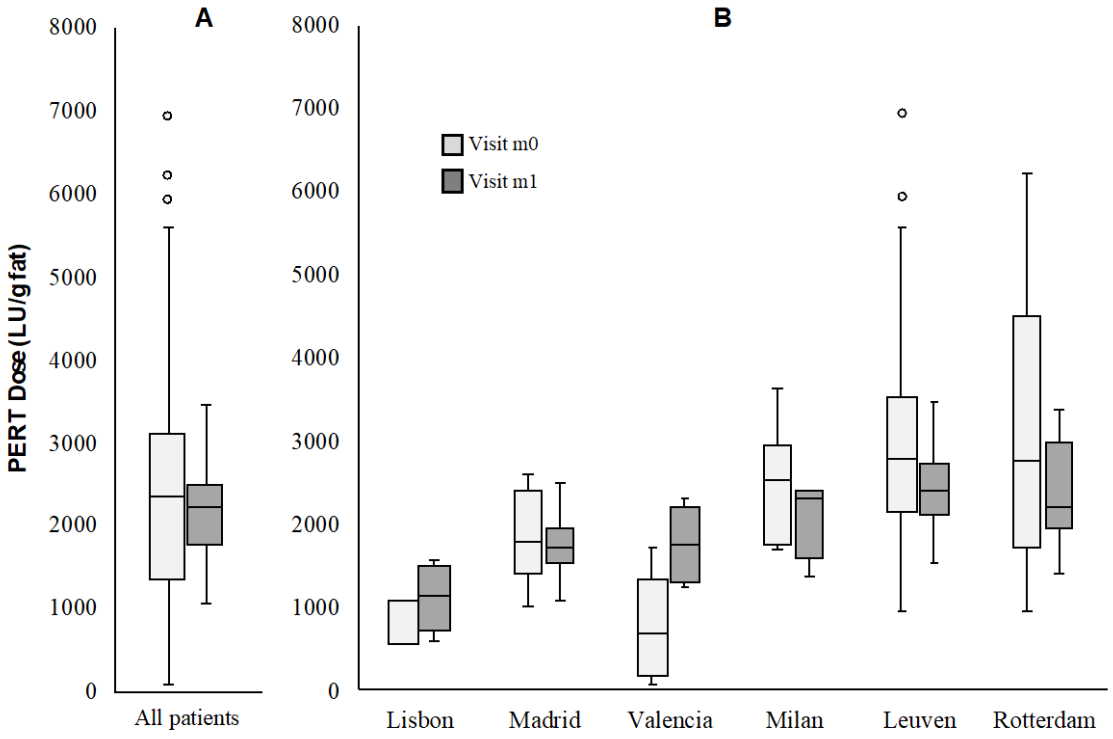

Figure 3. Change in PERT dose between visit 0 (regular dose) and visit 1 (optimal dose predicted by the app was enabled) expressed as lipase units per gram of dietary fat, considering all the centres combined (A) and displayed by centre (B). The recommended doses' range given by the predictive algorithm in the app was between 1000 and 4000 LU/g fat depending on the food characteristics and in function of the findings from *in vitro* data.

3.3. Change in fat absorption

Both at visit 0 and visit 1 the median CFA was higher than the clinical target of 90%), being respectively 96.9% (91.3, 98.7) and 96.7% (90.5, 98.8), with no statistically significant difference ($p>0.05$). When expressing fat absorption as grams of faecal fat/day, median values (1st and 3rd quartile) at visits 0 and 1 were similar ($p>0.05$): 2.6 g (0.9, 5.5) at visit 0 and 2.0 g (0.9, 5.4) at visit 1, the clinical target being <6 g (**Figure 4A**).

In patients with fat malabsorption (n=12) at visit 0 (CFA <90%) (**Figure 4B**), a significant improvement was seen: faecal fat decreased from 9.6 [5.9, 17.9] g to 4.5 [0.5,

8.1] g, p = 0.03) and CFA increased from 86.3 [82.5, 89.3] % to 94.0 [88.3, 98.8] %, p = 0.031). In this subgroup, median PERT doses did not change significantly between both study visits, being 2254 at visit 0 2090 LU/g fat at visit 1, but the interquartile ranges narrowed from 1545-3547 to 1463-2869 LU/g fat.

Overall, age, nutritional status parameters (z-scores of height, weight and BMI) or pulmonary function (FEV_1) were not associated with excreted fat nor CFA at visit 0.

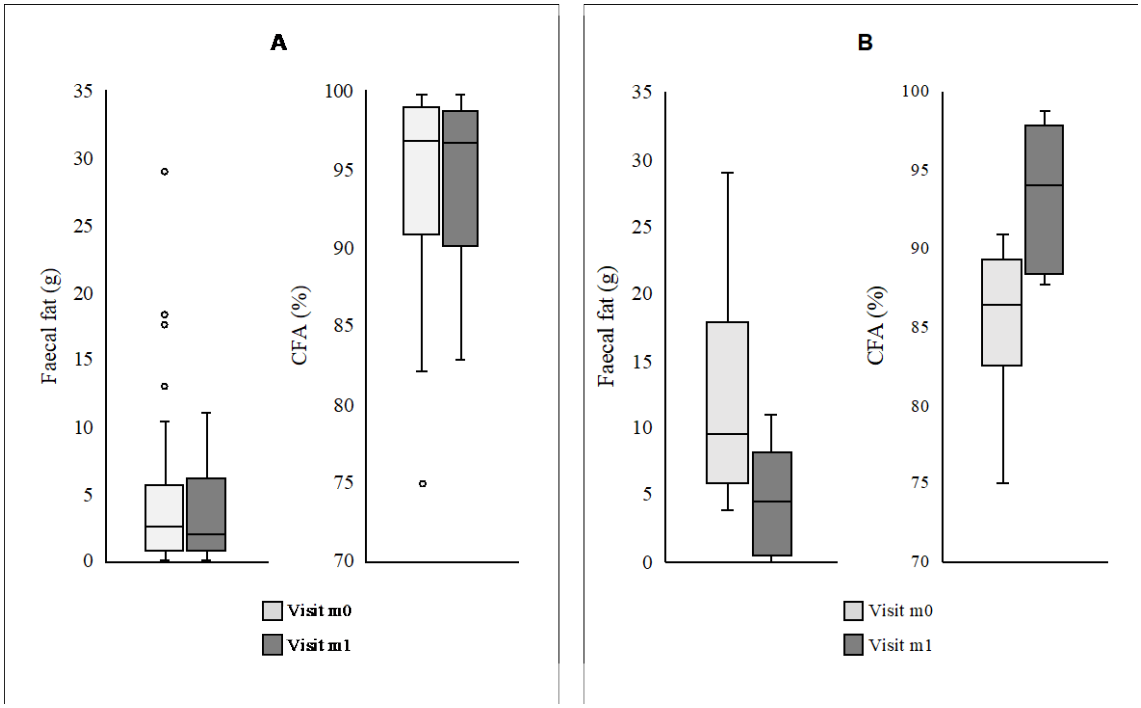

Figure 4. Change in fat absorption between visit 0 and visit 1, expressed as CFA % (coefficient of fat absorption) and grams of unabsorbed fat (g) considering the whole study sample (A) and a subset of patients (n=12) with the fat absorption parameters below the clinical target (CFA <90%) at the run-in visit (B).

4. DISCUSSION

This multicenter study in children with CF showed that a novel evidence-base PERT dosing method, based on incorporating food characteristics and delivered through an app, led to significant improvement of fat absorption in the children with poor baseline CFA. Although overall fat absorption parameters were already high at baseline and no

significant improvement was found, the results did show that the dosing of PERT became more precise (less variation) after using the app.

Regarding the use of PERT, a wide range of doses was seen at visit 0: low doses in some centres and high in others. Also, a high intra-centre variability was detected. This finding resembles the results obtained in our cross-sectional observational study conducted in 2016, in the same centres [33]. In that study, the centres of Lisbon, Madrid and Valencia reported median PERT doses below the guidelines' recommendation (2000-4000 LU/g fat) [8], strongly contrasting with those reported by the other centres (median dose range of 3805 and 7858 LU/g fat). This reflected the lack of a common criterion to establish PERT dose recommendations in Europe, existing up to date, which is similar to the situation in the US [34]. Altogether, the present intervention was efficient in modulating patients' self-adjustment of the PERT doses, bringing them closer to the recommendation of the assessed method, especially in the northern centres.

Despite of the change in PERT doses, CFA did not show a significant change between both study visits, as high baseline values made a further increase unlikely. These high baseline CFA values, suggest strong and effective clinical monitoring in the participating centres. This fact could have raised their awareness of the importance of adjusting the dose according to food characteristics, rather than supplying the same fixed dose for each type of meal. Interestingly, to our knowledge, there is no literature reporting similar high median CFA values; available studies reporting values below 85% [7, 35, 36]. In a previous pilot study with restricted food choices aimed at evaluating the currently applied evidence-based method to adjust PERT dose, median CFA was 90% [14]. In the present study, the same evidence-based method was evaluated in a slightly larger sample size (58 vs. 42 patients) and without dietary restrictions, and resulted in median CFA of 96.7%, which is comparable to healthy subjects [27]. This finding

documents the safety of the method and possibly, its validity to achieve satisfactory levels of fat absorption.

The most relevant finding of this study is, nonetheless, that patients with the lowest fat absorption at study baseline (n=12, CFA <90%), had a significant improvement in excreted fat and CFA. This result possibly suggests that the interventional tool could especially benefit patients with problems to achieve satisfactory values of fat absorption. However, the median daily PERT dose did not change in this subset of patients. This finding reinforces the message that not only the total dose in terms of "LU/g fat a day" is important for optimal fat absorption, but also the distribution of the dose in the different daily meals, according to the food matrix structure and the interaction with other nutrients [13]. Other interventional studies aimed at improving fat absorption in CF also, only reported benefits in patients with severe fat malabsorption [37]. Similarly, in the clinical trial in which this substudy was conducted, the main outcome parameter, i.e., gastrointestinal-related quality of life, showed greater improvement in patients with the worst baseline evaluation [29]. The clinical relevance of the improved fat absorption in these patients could have several implications besides reduced energy loss, as undigested fat causes gastrointestinal symptoms [4], malabsorption of fat-soluble vitamins [8], and could imply dysbiosis in the gut microbiota [38].

Summing up, the strength of this study is the novelty of the intervention, as to our knowledge no other attempt to establish an evidence-based method to adjust PERT dose has been reported. In addition, the present study adds to the literature about PERT use and fat absorption indicators in a relatively large and multi-centric series of patients with CF. The study also has some limitations. The first was inherent to the nature of the results at baseline (fat absorption), which were already better than the aimed target value after the intervention. Another limitation is the complexity of the malabsorption mechanisms,

which could have prevented fat from being absorbed despite having been optimally digested [38]. Some studies suggest intraluminal mechanisms, such as impossibility to absorb long-chain fatty acids (i.e., most of the dietary lipids) due to low bile salts concentration that impede micellation, as the reason for fat malabsorption in CF [39,40]. Other *in vitro* digestion studies in CF concluded that indeed bile salts and also other intraluminal factors such as intestinal pH are major determinants of the efficacy of the enzyme supplement [21, 41]. The complexity of malabsorption is clear when inherent-to-food factors, such as food matrix structure or interactions among macronutrients, are considered [13, 21-25].

When looking into other studies targeting improvement of fat malabsorption, a few successful attempts have been seen up to now. For example, the use of proton pump inhibitors to improve alkalinisation of the intestinal fluids and activity of PERT has been a recurrent subject of study. However, a recent systematic review concluded that there is limited evidence supporting improvement in fat absorption [42]. A more recent approach relates to the use of microbial-origin enzymatic supplements, the most recent study in the field showing improvement compared to placebo, but achieving CFA values below the clinical target [43]. Some of the most up-to-dated strategies include the use of an in-line digestive cartridge that hydrolyses fat in enteral formula [44]. The treatment is effective in improving long-term fat absorption, but as restricted to patients in enteral nutrition regimes, its scope is limited. Other promising and novel interventions propose the supplementation with readily absorbable structured lipid [4, 37].

Based on the results of present study, the recommended range of PERT dose could be enlarged to 1000-4000 LU/g fat. The tested evidence-based method relied on recommended doses for individual foods of either 1000, 2000, 3000 or 4000 LU/g fat. As fat absorption indicators resulted in median 96.7% CFA, this suggestion seems

reasonable. However, to provide the level of evidence needed for including this recommendation in the clinical guidelines, this method should be further tested. Longer periods of observation including more frequent fat absorption measurements and inclusion of more patients with baseline poor fat absorption would be needed to confirm improved CFA, gastrointestinal symptoms and possibly nutritional status and growth or other markers of absorption including vitamin levels.

In conclusion, adhering to the evidence-based PERT dosing method allowed for achieving high CFA results in children with CF. Interestingly, patients with the poorest fat absorption at baseline, did show an improvement after using the evidence-based method, which encourages further research.

ACKNOWLEDGEMENTS

We acknowledge the support of the MyCyFAPP Project consortium. We especially thank the participation and the effort of the patients involved in the study and their families. This work was fully funded by the European Union and the Horizon 2020 Research and Innovation Framework Programme (PHC-26-2014 call Self management of health and disease: citizen engagement and mHealth) under grant number 643806.

CONFLICT OF INTEREST

Authors have nothing to disclose.

5. REFERENCES

1. C. Colombo, J. Littlewood. The implementation of standards of care in Europe: state of the art. J Cyst Fibros, 10 (2011), pp. S7–S15.

2. R.D. Baker. Assessing exocrine pancreatic function: When the best test is not possible. J Pediatr Gastroenterol Nutr, 56 (2013), pp. 116-117.

3. D Borowitz, P.R. Durie, L.L. Clarke, S.L. Werlin, C.J. Taylor, J. Semler, *et al.* Gastrointestinal outcomes and confounders in cystic fibrosis. J Pediatr Gastr Nutr, 41 (2005), pp. 273-285.

4. N.J. Rowbotham, S. Smith, P.A. Leighton, O.C. Rayner, K. Gathercole, Z.C. Elliott, *et al*. The top 10 research priorities in cystic fibrosis developed by a partnership between people with cf and healthcare providers. Thorax, 73 (2018), pp. 388-390.

5. E.H. Yen, H. Quinton, D. Borowitz. Better nutritional status in early childhood is associated with improved clinical outcomes and survival in patients with cystic fibrosis. J Pediatr, 162 (2013), pp. 530–535

6. A.L. Stephenson, L.A. Mannik, S. Walsh, M. Brotherwood, R. Robert, P.B. Darling, *et al*. Longitudinal trends in nutritional status and the relation between lung function and BMI in cystic fibrosis: a population-based cohort study. Am J Clin Nutr, 97 (2013), pp. 872-877.

7. J.W. Woestenenk, C.K. Van Der Ent, R.H.J. Houwen. Pancreatic enzyme replacement therapy and coefficient cystic fibrosis. Pancreatology, 61 (2015), pp. 355-360

8. D. Turck, C.P. Braegger, C. Colombo, D. Declercq, A. Morton, R. Pancheva, *et al*. ESPEN-ESPGHAN-ECFS guidelines on nutrition care for infants, children, and adults with cystic fibrosis. Clin Nutr, 35 (2016), pp. 557-577.

9. J.E. Domínguez–Muñoz. Chronic pancreatitis and persistent steatorrhea: what is the correct dose of enzymes?. Clin Gastroenterol Hepatol, 9 (2011), pp. 541-546.

10. A.R. Smyth, S.C. Bell, S. Bojcin, M. Bryon, A. Duff, P. Flume, *et al*. European cystic fibrosis society standards of care: best practice guidelines. J Cyst Fibros, 13 (2014), pp. S23-S42.

11. R. Sutherland, T. Katz, V. Liu, J. Quintano, R. Brunner, C.W. Tong, *et al*. Dietary intake of energy-dense, nutrient-poor and nutrient-dense food sources in children with cystic fibrosis. J Cyst Fibros, 17 (2018), pp. 804-810.

12. J. Calvo-Lerma, A. Asensio-Grau, A. Heredia, A. Andrés. Lessons learnt from MyCyFAPP Project: Effect of cystic fibrosis factors and inherent-to-food properties on lipid digestion in foods. Food Res Int, 133 (2020) pp. 109198

13. J. Calvo-Lerma, V. Fornés-Ferrer, A. Heredia, A. Andrés. In vitro digestion of lipids in real foods: influence of lipid organization within the food matrix and interactions with nonlipid components. J Food Sci, 83 (2018), pp. 2629-2637.

14. Q. Guo, A. Ye, N. Bellissimo, H. Singh, D. Rousseau, D. Modulating fat digestion through food structure design. Prog Lipid Res, 68 (2017), pp. 109-118.

15. M. Hiolle, V. Lechevalier, J. Floury, N. Boulier-Monthéan, C. Prioul, D. Dupont, *et al*. In vitro digestion of complex foods: How microstructure influences food disintegration and micronutrient bioaccessibility. Food Res Int, 128 (2020), pp. 108817.

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

17. D. Gelfond, C. Ma, J. Semler, D. Borowitz. Intestinal pH and gastrointestinal transit profiles in cystic fibrosis patients measured by wireless motility capsule. Dig Dis Sci, 58 (2013), pp. 2275–2281.

18. P.J. Robinson, A.L. Smith, P.D. Sly. Duodenal pH in cystic fibrosis and its relationship to fat malabsorption. Dig Dis Sci, 35 (1990), pp. 1299-1304.

421 19. J.T. Harries, D.P. Muller, J.P. McCollum, A. Lipson, E. Roma, A.P. Norman.
422 Intestinal bile salts in cystic fibrosis: Studies in the patient and experimental animal. Arch
423 Dis Child, 54 (1979), pp. 19–24.
424 20. L. Humbert, D. Rainteau, N. Tuvignon, C. Wolf, P. Seksik, R. Laugier, F.
425 Carrière. Postprandial bile acid levels in intestine and plasma reveal altered biliary
426 circulation in chronic pancreatitis patients. J Lipid Res, 59 (2018), pp. 2202–2213.
427 21. A. Asensio-Grau, I. Peinado, A. Heredia, A. Andrés. Effect of cooking
428 methods and intestinal conditions on lipolysis, proteolysis and xanthophylls
429 bioaccessibility of eggs. J Funct Foods, 46 (2018), pp. 579-586.
430 22. A. Asensio-Grau, J. Calvo-Lerma, A. Heredia, A. Andrés. Fat digestibility in
431 meat products: influence of food structure and gastrointestinal conditions. Int J Food Sci
432 Nutr, 70 (2019), pp. 530-539.
433 23. A. Asensio-Grau, I. Peinado, A. Heredia, A. Andrés. In vitro study of cheese
434 digestion: Effect of type of cheese and intestinal conditions on macronutrients
435 digestibility. LWT, 113 (2019) pp. 108278.
436 24. C. Paz-Yépez, I. Peinado, A. Heredia, A. Andrés. Influence of particle size
437 and intestinal conditions on in vitro lipid and protein digestibility of walnuts and peanuts.
438 Food Res Int, 119 (2019) pp. 951-959.
439 25. C. Paz-Yépez, I. Peinado, A. Heredia, A. Andrés. Lipids digestibility and
440 polyphenols release under in vitro digestion of dark, milk and white chocolate. J Funct
441 Foods, 52 (2019), pp. 196-203.
442 26. J. Calvo-Lerma, V. Fornés-Ferrer, I. Peinado, A. Heredia, C. Ribes-Koninckx,
443 A. Andrés. A first approach for an evidence-based in vitro digestion method to adjust
444 pancreatic enzyme replacement therapy in cystic fibrosis. PLoS One, 14 (2019), pp.
445 e0212459

446 27. J. Calvo-Lerma, J. Hulst, M. Boon, C. Colombo, E. Masip, M. Ruperto, *et al.*
447 Clinical validation of an evidence-based method to adjust Pancreatic Enzyme
448 Replacement Therapy through a prospective interventional study in paediatric patients
449 with Cystic Fibrosis. PLoS One, 14 (2019), pp. e0213216.
450 28. A. Martinez-Millana, A. Zettl, J. Floch, J. Calvo-Lerma, J.L. Sevillano, C.
451 Ribes-Koninckx, *et al*. The potential of self-management mhealth for pediatric cystic
452 fibrosis: mixed-methods study for health care and app assessment. JMIR mHealth and
453 uHealth, 7 (2019), pp. e13362.
454 29. M. Boon, J. Calvo-Lerma, I. Claes, T. Havermans, I. Asseiceira, A.
455 Bulfamante, *et al*. Use of a mobile application for self-management of pancreatic enzyme
456 replacement therapy is associated with improved gastro-intestinal related quality of life
457 in children with Cystic Fibrosis. J. Cyst Fibros 19 (2020) pp. 562-568.
458 30. J. Calvo-Lerma, M. Roca, M. Boon, C. Colombo, B. de Koning, V. Fornés-
459 Ferrer, et al. (2020). Association between faecal pH and fat absorption in children with
460 cystic fibrosis on a controlled diet and enzyme supplements dose. J Pediatr Res, In Press
461 (2020) https://doi.org/10.1038/s41390-020-0860-3
462 31. R.J. Kuczmarski, C.L. Ogden, S.S. Guo, L.M. Grummer-Strawn, K.M. Flegal
463 Z. Mei, *et al*. 2000 CDC growth charts for the United States: methods and development.
464 National Center for Health Statistics. Vital Health Stat, 246 (2002), pp. 1-190.
465 32. P.H. Quanjer, S. Stanojevic, T.J. Cole, X. Baur, G.L. Hall, B.H. Culver, *et al.*
466 Multiethnic reference values for spirometry for the 3-95-yr age range: the global lung
467 function 2012 equations. Eur Respir J, 40 (2012), pp.1324-1343.
468 33. J. Calvo-Lerma, J. Hulst, I. Asseiceira, I. Claes, M. Garriga, C. Colombo, *et*
469 *al*. Nutritional status, nutrient intake and use of enzyme supplements in paediatric patients

with Cystic Fibrosis; a European multicentre study with reference to current guidelines. J Cyst Fibros, 16 (2017), pp. 510-518.

34. D. Gelfond, S.L. Heltshe, M. Skalland, J.E. Heubi, M. Kloster, D. Leung, *et al.* Pancreatic Enzyme Replacement Therapy use in infants with cystic fibrosis diagnosed by newborn screening. J Pediatr Gastroenterol Nutr, 66 (2018), pp. 657-663.

35. D. Borowitz, M.W. Konstan, A. O'Rourke, M. Cohen, L. Hendeles, F.T. Murray. Coefficients of fat and nitrogen absorption in healthy subjects and individuals with cystic fibrosis. The Journal of Pediatric Pharmacology and Therapeutics, 12 (2007), pp. 47-52.

36. J. Calvo-Lerma, S. Martínez-Barona, E. Masip, V. Fornés, C. Ribes-Koninckx. Pancreatic enzyme replacement therapy in cystic fibrosis: dose, variability and coefficient of fat absorption. Rev Esp Enf Dig, 109 (2017), pp. 684-689.

37. V.A. Stallings, A.M. Tindall, M.R. Mascarenhas, A. Maqbool, J.I. Schall. Improved residual fat malabsorption and growth in children with cystic fibrosis treated with a novel oral structured lipid supplement: A randomized controlled trial. PLoS One, 15(2020), pp. e0232685.

38. J.M. Ridlon, D.J. Kang, P.B. Hylemon. Bile salt biotransformations by human intestinal bacteria. J Lip Res, 47 (2006), pp. 241-259.

39. M. Kalivianakis, H.J. Verkade. The mechanisms of fat malabsorption in cystic fibrosis patients. Nutrition, 15 (1999), pp. 167-169.

40. N. Peretti, V. Marcil, E. Drouin, E. Levy. Mechanisms of lipid malabsorption in Cystic Fibrosis: the impact of essential fatty acids deficiency. Nutr Metab, 2 (2005), pp. 11.

41. J. Calvo-Lerma, V. Fornés-Ferrer, A. Heredia, A. Andrés. In vitro digestion models to assess lipolysis: The impact of the simulated conditions of gastric and intestinal pH, bile salts and digestive fluids. Food Res Int, 125 (2019), pp. 108511.

42. S.M. Ng, A.J. Francini. Drug therapies for reducing gastric acidity in people with cystic fibrosis. Cochrane Database Syst Rev, 4 (2012).

43. J.E. Heubi, D. Schaeffer, R.C. Ahrens, N. Sollo, S. Strausbaugh, G. Graff, e*t al*. Safety and efficacy of a novel microbial lipase in patients with exocrine pancreatic insufficiency due to cystic fibrosis: a randomized controlled clinical trial. J Pediatr, 176 (2016), pp. 156-161.

44. J. Stevens, C. Wyatt, P. Brown, D. Patel, D. Grujic, S.D Freedman. Absorption and safety with sustained use of RELiZORB evaluation (ASSURE) study in patients with cystic fibrosis receiving enteral feeding. J Pediatr Gastr Nutr, 67 (2018), pp. 527-532