Ready-to-Use Supplemental Food for Nutritional Supplementation in Cystic Fibrosis

Ryan T Pitman, Melody Mui, Peter H Michelson, and Mark J Manary

Department of Pediatrics, Washington University in St. Louis, St. Louis, MO

ABSTRACT

Undernutrition is common in cystic fibrosis (CF) and is correlated with long-term outcomes, yet current nutritional interventions have not demonstrated consistent improvements in energy intake, and subsequently, growth. Development of novel nutritional interventions to increase energy intake is essential to improve clinical outcomes of individuals with CF. Ready-to-use supplemental food (RUSF) is a modifiable, inexpensive, palatable, safe, and nutrient-dense food for treatment or prevention of acute malnutrition in developing countries. Utilizing a linear-programming tool we identified 6 RUSF formulations with sufficient nutrient density (495 kcal/100 g), protein, and fat for children with CF. Palatability was established by a taste-trial and affirmed by a 2-wk tolerability assessment that demonstrated consistent consumption and tolerance of the RUSF. Although preliminary, this study demonstrates the potential for developing RUSF as a nutritional supplement for increasing energy intake in children with CF.

Introduction

Growth faltering is common in children with chronic disease (1) and is predictive of future morbidity or mortality (2–4). In cystic fibrosis (CF), favorable early-in-life growth is related to enhanced pulmonary function later in life, but achieving recommended energy, protein, and fat intake is often difficult (5–9). Despite significant improvements over the last 3 decades, 24% of children with CF are stunted and up to 25% are underweight (8).

Interventions to improve dietary intake in CF, including behavioral techniques and liquid supplements, promote modest weight gain, but are limited by personnel requirements, distaste for the supplement, or the volume required to be ingested (10). Current liquid nutritional supplement products are not designed for the unique nutritional needs of children with CF because the contributions of fat and protein fail to meet current nutrition recommendations (8, 11). Further, liquid nutritional supplements provide low amounts of α-linolenic acid (18:3n–3), an essential fatty acid commonly deficient in children with CF (8, 12, 13). In addition, energy from liquid supplements may replace that from the habitual diet, resulting in no net gain in energy intake (14). Development of new options to enhance protein and energy intake could well improve growth and improve long-term outcomes.

Ready-to-use supplemental food (RUSF) is a palatable, protein, and energy-dense lipid paste comprised of peanut butter, milk powder, and oil, initially developed for children and adults in resource-poor settings for the treatment of acute malnutrition (15). RUSF formulation and nutrient composition are easily modifiable dependent on disease-specific nutrition requirements, ingredient restrictions, and cultural preferences (16).

In this study, we created an appropriately formulated RUSF for CF (RUSF-CF) and tested the hypothesis that RUSF-CF was a palatable and acceptable food for underweight children with CF.
Methods

RUSF-CF development
Based on a thorough consideration of the CF literature we concluded that RUSF-CF must contain 40% lipid and 15% protein at a minimum (8, 9). Using current guidelines for developing ready-to-use therapeutic food and an Excel-based (Microsoft) linear-programming tool, previously developed in our center, we identified and tested candidate RUSF formulations (15–17). The linear-programming tool pairs a computer database of candidate ingredients and their nutrient composition with a computational algorithm that considers programmed inputs including cost, ingredient availability, and desired nutritional specifications to provide a candidate formula. Upon identifying ingredients readily available in North America and inputting disease-specific nutrition constraints, an iterative process was pursued in which candidate formulations were identified and assessed for production ability, ingredient interactions, and predicted sensory experience. Potential formulations were then prepared on the bench-top level in our food lab at Washington University and underwent subjective assessment of texture and palatability (16). A preliminary formulation including peanut paste, milk powder, oat flour, canola oil, and sugar was subsequently modified by differing the base legume (soy or peanut) and flour source (oat milk powder, oat flour, canola oil, and sugar) was subsequently modified. Ultimately, 3 candidate RUSF formulations with similar energy density and protein, fat, and carbohydrate content were identified (Table 1). Three flavored variations of the peanut-RUSF-CF (cinnamon, cocoa, and cinnamon and cocoa) were developed without significant impact on nutritional composition.

RUSF-CF production
Candidate RUSF-CF formulations were prepared as described previously (15). Each RUSF was formulated to include a mono- and diglyceride emulsifier (T-180, Caravan Foods) at ~2%. Briefly, the lipid emulsifier and oil were mixed while heated until the powdered emulsifier was fully dissolved. A subset of the dry ingredients (milk powder, sugar, oat flour, and peanut paste) were blended, subsequently combined with the emulsifier oil, and then homogenized. The RUSF was prepared in a certified food lab under clean conditions and each batch was subject to microbial analysis (Eurofins) per World Food Program specifications (18). Prepared RUSF-CF was packaged in induction-sealed 400-g polyethylene terephthalate jars with an anticipated shelf life of 4 mo (15).

Ethical considerations
All study procedures were approved by the Institutional Review Board at Washington University in St. Louis. Verbal assent was obtained from all subjects with written consent from their parents/guardians.

Palatability and tolerability assessment
Palatability assessments were performed in the Washington University Pediatric CF clinic. Participants were provided a 10-g sample of each of the 6 RUSF-CF variations in a uniform sequential order. After each tasting, they provided a dichotomous determination of palatability (yes/no), and after trying all 6 RUSF-CF versions, provided a rating in order of preference (with their preferred version ranked first) (19).

Tolerability of sustained consumption of RUSF-CF and assessment of adverse effects were determined through a 2-wk crossover study. The 2 lead RUSF-CF candidates from the palatability assessment were identified and used to determine tolerability.

Subjects with CF and pancreatic insufficiency between the ages of 2 and 12 y with a BMI or weight-for-age percentile <75th centile were recruited from the Washington University CF clinic. Patients with CF-related diabetes, CF-related liver disease, or allergies to RUSF-CF ingredients were excluded. Anthropometric measurements including weight with minimal clothing to the nearest 100 g, height to the nearest 1 cm, and midupper arm circumference to the nearest 1 mm were obtained at enrollment. Triceps and subscapular skinfold measurements were taken with calipers to the nearest 1 mm.

Subjects were randomly assigned into 2 cohorts and provided 1 RUSF-CF for 1 wk, after which they received the crossover lead candidate for 1 wk, as described previously (20). A crossover lead design was utilized to minimize the influence of RUSF-CF order on consumption or assessment of likability. Random assignment was achieved by an Excel-based (Microsoft) random group generator. Individual participants received RUSF-CF intake recommendations equivalent to ~20% of daily estimated energy requirements (EERs) (in kilocalories; to the nearest 100 kcal for ease of measurement) (13). Subjects were instructed to continue their current nutritional regimen including provider-prescribed pancreatic enzyme replacement therapy and fat-soluble vitamin supplements. Families were allowed to present the RUSF-CF in any manner the child preferred, such as plain, on bread/toast, or with fruit.

RUSF-CF was provided in 400-g jars. Families were also provided standard tablespoons for measurement of RUSF-CF. Laboratory analysis determined 1 tablespoon to be equivalent to 20 g of RUSF-CF. RUSF-CF was provided at enrollment and through the US mail to arrive at the 1-wk crossover point.

Daily intake and gastrointestinal symptoms were assessed at the end of each study week. Overall acceptability was determined by a 5-point hedonic scale (19). Final RUSF-CF preference and repeat anthropometry were obtained at the end of the 2-wk period.

Statistical analysis
Anthropometric indexes were calculated using Anthro version 3.1 (WHO) based on the WHO’s 2006 Child Growth Standards. Descriptive statistics are presented as mean ± SD for continuous variables. Wilcoxon’s Signed Rank test was used for continuous variables. All statistics were performed using SPSS statistics version 24 (IBM).

Results
Prepared peanut-RUSF-CF provided 495 kcal, 18.6 g protein, and 28.9 g lipid per 100 g. Fat comprised 51% of total energy, whereas protein contributed 15% of total energy. The α-linolenic acid (1.3 g) exceeded current recommendations for daily adequate intake of omega-3 polyunsaturated fatty acids (13). The 3 base formulations differed minimally in calculated energy and protein composition (Table 1), yet had more protein and fat than standard ready-to-use therapeutic food (21). In comparison with current commercially available nutrition supplements, the peanut-RUSF-CF contained more protein and lipid per serving (Table 2) (22–25).
Nine children with CF (age 3–10 y) and a mean BMI percentile of 39.2% ± 26.6% participated in the palatability assessment. Eight of 9 children completed all tastings. Each formulation was found to be palatable by approximately half (4.9 ± 0.7) of the participants. The RUSF-CF formulations were then ranked in terms of preference, with the cinnamon peanut-RUSF-CF and regular peanut-RUSF-CF receiving the highest aggregate rankings followed by the cocoa peanut-RUSF-CF, buckwheat-RUSF-CF, soy-RUSF-CF, and cinnamon/cocoa peanut-RUSF-CF.

Separately, to assess tolerability, 10 subjects (age 3–12 y, 4 female) with a mean BMI percentile of 50.47% ± 28.2%, 4 with gastrostomies providing 58.7% ± 18.8% EER in enteral nutrition, and 4 prescribed liquid nutritional supplements providing 13.5% ± 0.4% EER were recruited. Cohorts were combined for analysis of intake and likability because no significant differences in RUSF-CF intake, likability, or side effects were detected between cohorts. Over the 2-wk study, mean BMI z score (−0.35 ± 1.0 to −0.21 ± 1.0; P = 0.043) and weight (29 ± 11 kg to 30.2 ± 12 kg; P = 0.042) increased, without significant impacts on weight-for-age z score, midupper arm circumference, and triceps or subscapular skinfold measurements.

Participants consumed RUSF-CF on 52% ± 36% of study days, with a mean estimated intake of 170 ± 173 kcal/d. Mean intake for children without gastrostomies was 203 ± 186 kcal/d. RUSF was consumed in a variety of manners including plain (55% of participants), on bread or crackers (55%), combined into a milkshake or smoothie (22%), or consumed with fruit (22%). Over the course of 2 wks, subjects collectively reported 1 episode of diarrhea and 1 episode of abdominal pain. No significant differences were found between the 2 RUSF-CF types in overall consumption, median (IQR) hedonic scale ranking [4 (2.25 – 4) compared with 4 (2 – 4)], or side effects.

### TABLE 1  Ingredient composition of unflavored candidate RUSFs with macronutrients and selected micronutrients, per 100-g serving

| Ingredients, g/100 g | Peanut/oat | Peanut/buckwheat | Soy/oat | Standard RUTF |
|----------------------|------------|------------------|--------|--------------|
| Legume, peanut paste | 29.0       | 29.0             | —      | 27.0         |
| Roasted full-fat soy flour | —         | —                | 17.1   | —            |
| Nonfat dry milk     | 14.1       | 14.1             | 15.0   | 15.8         |
| Palm oil            | —          | —                | —      | 2.9          |
| High oleic sunflower oil | —        | —                | 9.3    | —            |
| White sugar         | 19.0       | 18.0             | 18.0   | 26.0         |
| Oat flour           | 4.9        | —                | 9.7    | —            |
| Buckwheat flour     | —          | 7.3              | —      | —            |

| Nutrients, per 100 g | Peanut/oat | Peanut/buckwheat | Soy/oat | Standard RUTF |
|----------------------|------------|------------------|--------|--------------|
| Energy, kcal         | 495.0      | 490.9            | 495.0  | 500.0        |
| Protein, g           | 18.5       | 18.2             | 17.3   | 12.8         |
| Lipid, g             | 28.3       | 28.2             | 28.9   | 30.3         |
| n-3, g               | 1.3        | 1.3              | 1.7    | —            |
| n-6, g               | 2.8        | 2.7              | 5.2    | —            |
| Carbohydrate, g      | 42.1       | 42.3             | 44.2   | 45.0         |
| Fiber, g             | 0.5        | 0.7              | 2.7    | —            |
| Sodium, mg           | 160.4      | 153.0            | 146.3  | 165.0        |
| Calcium, mg          | 385.1      | 367.9            | 375.7  | 302.0        |
| Phosphorus, mg       | 407.6      | 393.1            | 392.5  | 343.0        |
| Potassium, mg        | 750.5      | 746.6            | 874.3  | 1171         |
| Magnesium, mg        | 96.0       | 104.1            | 110.0  | 80.0         |
| Iron, mg             | 1.5        | 1.6              | 1.5    | 10.3         |
| Zinc, mg             | 2.0        | 1.9              | 2.1    | 11.8         |
| Copper, mg           | 0.2        | 0.2              | 0.5    | 1.5          |
| Selenium, µg         | 8.0        | 8.1              | 8.7    | 28.0         |
| Vitamin A, RAE       | 1.7        | 1.7              | 2.6    | 0.8          |
| Vitamin D, µg        | 0.0        | 0.0              | 0.0    | 14.0         |
| Vitamin E, mg        | 5.4        | 5.4              | 6.8    | 18.4         |
| Vitamin K, µg        | 10.1       | 10.1             | 23.4   | 14.4         |
| Thiamin, mg          | 0.3        | 0.3              | 0.3    | 0.5          |
| Riboflavin, mg       | 0.5        | 0.5              | 0.6    | 1.5          |
| Vitamin C, mg        | 2.0        | 1.9              | 1.8    | 46.0         |
| Vitamin B-6, mg      | 0.3        | 0.3              | 0.2    | 0.5          |
| Vitamin B-12, µg     | 1.2        | 1.1              | 1.1    | 1.5          |
| Folic acid, µg       | 0.0        | 0.0              | 0.0    | 184.0        |
| Niacin, mg           | 4.8        | 5.1              | 0.9    | 4.6          |
| Pantothenic acid, mg | 1.1        | 1.0              | 1.3    | 2.8          |

1 RAE, retinal activity equivalents; RUSF, ready-to-use supplemental food; RUTF, ready-to-use therapeutic food.
TABLE 2  Comparison of peanut RUSF macronutrient and select micronutrient composition with commercially available nutritional supplements1

|                        | Peanut RUSF | Pediasure | Ensure | Boost | Scandishake |
|------------------------|-------------|-----------|--------|-------|-------------|
| Volume/weight          | 100 g       | 237 mL    | 237 mL | 237 mL| 85 g2       |
| Kcal                   | 495         | 240       | 220    | 240   | 425         |
| Kcal from fat          | 253         | 80        | 50     | 35    | 46          |
| Saturated fat, g       | 1.3         | 1         | 1      | 1     | 9           |
| Protein, g             | 18.6        | 7         | 9      | 10    | 4           |
| Fat, g                 | 28.5        | 9         | 6      | 4     | 21          |
| Carbohydrate, g        | 42          | 33        | 33     | 41    | 55          |
| Sugar, g               | 19          | 12        | 15     | 25    | 7           |
| Cholesterol, mg        | 5.8         | 5         | <5     | 10    | —           |
| Sodium, mg             | 160         | 90        | 190    | 150   | 119         |
| Potassium, mg          | 750         | 350       | 390    | 460   | 484         |
| Dietary fiber, g       | 0.5         | <1        | 1      | 0     | 0           |

1RUSF, ready-to-use supplemental food.
2Scandishake is a powder that must be dissolved in 8 oz (237 ml), liquid.

Discussion

In this preliminary study, we demonstrate the feasibility of developing RUSF-CF as a nutritional supplement for children with CF. To our knowledge, this is the first use of RUSF for nutritional supplementation in a chronic pediatric disease in the developed world.

Other products are available or have previously been studied as nutritional supplements in CF (26–28) with some benefit in increasing weight. However, RUSF-CF may yield benefits not previously provided. RUSF is easily consumed as a part of a standard meal without replacing energy from other elements of the meal. RUSF-CF can be consumed in a variety of presentations and is found to be palatable by most children. Although currently available nutritional supplements are generally found to be palatable by children with CF, and we present no formal comparison of RUSF-CF palatability with other supplements, many children experience supplement fatigue and may appreciate multiple options for energy supplementation. A 100-g serving of the peanut-RUSF-CF provides nearly 24.7% kcal, 18% of recommended protein intake (100 g/d), and 31.9% of recommended lipid intake (89 g/d) for a 2000-kcal diet (8, 11). This represents an increase in nutritional density from currently available nutritional supplements, an important consideration because intakes of protein (12% of dietary calories) and fat (34–35% of dietary calories) are persistently inadequate in children with CF despite recommendations for dietary protein and fat to comprise 20% and 40% of total calories, respectively (6, 8, 11, 29).

Effective supplementation requires increasing total energy intake and not mere substitution of supplement calories for calories obtained from other foods (14, 30). Previous literature has not demonstrated consistently increased energy intake from nutrient supplements alone (5, 10, 27, 30). Preliminary results for our acceptability study suggest that intake of RUSF-CF constitutes nearly 10% of dietitian-recommended daily energy intake, in addition to the participant’s regularly prescribed nutrition therapies. Although we did not record each subject’s total energy intake during this study, consistent intake of 5–10% of calories in excess of energy expenditure could lead to significant sustained improvements in growth (27).

This study has many limitations. Although it is theoretically possible to use RUSF for supplemental nutrition in a variety of disease states, we urge caution with extending this process to other diseases without careful consideration of the specific nutritional needs of each population. The demonstration population for palatability and tolerability was small and was comprised of only children with CF. Taste or texture preferences may differ in other disease states related to disease complications, therapies, or medications. Further, the acceptability study was short in duration, taste preferences may change over periods of sustained intake, and a liquid supplement was not provided as a control to compare palatability or tolerability.

In addition, the concentrations of sodium, although similar to other nutritional supplements (Table 2), and potassium in CF-RUSF were not sufficient to meet daily intake recommendations because CF-RUSF was not intended for electrolyte supplementation. Nutrition therapy in CF requires close medical monitoring and long-term studies including pilot and controlled trials are required to validate this approach for developing new nutritional supplements for children with CF.

Outcomes for patients with CF have improved significantly over the past 2 decades (7). Yet, innovations to enhance nutritional status have been identified as a vital step in advancing care for children with chronic illness, especially CF (8). In this study, we demonstrate the development and initial testing of a therapeutic food for nutritional supplementation in children with a chronic illness.

Acknowledgments

The authors’ responsibilities were as follows—RTP and MM: conducted the research; RTP, PHM, and MJM: analyzed the data and prepared the manuscript; MJM: has primary responsibility for final content; and all authors: designed the research and read and approved the final manuscript.

References

1. Sevilla WMA. Nutritional considerations in pediatric chronic disease. Pediatr Rev 2017;38(8):343–52.
2. Yang CH, Perumpail BJ, Yoo ER, Ahmed A, Kerner JA Jr. Nutritional needs and support for children with chronic liver disease. Nutrients 2017;9(10):1127.
3. Forbes A, Escher J, Hebuterne X, Kleck S, Krznaric Z, Schneider S, Shamir R, Stardelova K, Wierdsma N, Wiskin AE, et al. ESPEN guideline: clinical nutrition in inflammatory bowel disease. Clin Nutr 2017;36(2):321–47.

4. Iorember FM. Malnutrition in chronic kidney disease. Front Pediatr 2018;6:161.

5. Powers SW, Patton SR, Byars KC, Mitchell MJ, Jelalian E, Mulvihill MM, Hovell MF, Stark LJ. Caloric intake and eating behavior in infants and toddlers with cystic fibrosis. Pediatrics 2002;109(5):E75.

6. Filigino SS, Robson SM, Szczesniak RD, Chamberlin LA, Baker MA, Sullivan SM, Kroner J, Powers SW. Macronutrient intake in preschoolers with cystic fibrosis and the relationship between macronutrients and growth. J Cyst Fibros 2017;16(4):519–24.

7. Cystic Fibrosis Foundation Patient Registry. 2017 Annual Data Report. Bethesda, MD: Cystic Fibrosis Foundation; 2018.

8. Turck D, Braegger CP, Colombo C, Declercq D, Morton A, Pancheva R, Robberecht E, Stern M, Strandvik B, Wolfe S, et al. ESPEN-ESPGHAN-ECFS guidelines on nutrition care for infants, children, and adults with cystic fibrosis. Clin Nutr 2016;35(3):557–77.

9. Stallings VA, Stark LJ, Robinson KA, Feranchak AP, Quinton H. Clinical Practice Guidelines on Growth and Nutrition Subcommittee, Ad Hoc Working Group. Evidence-based practice recommendations for nutrition-related management of children and adults with cystic fibrosis and pancreatic insufficiency: results of a systematic review. J Am Diet Assoc 2008;108(5):832–9.

10. Woestenenk JW, Casteljns SJ, van der Ent CK. Houwen RH. Nutritional intervention in patients with cystic fibrosis: a systematic review. J Cyst Fibros 2013;12(2):102–15.

11. Engelen MP, Com G, Deutz NE. Protein is an important but undervalued macronutrient in the nutritional care of patients with cystic fibrosis. Curr Opin Clin Nutr Metab Care 2014;17(6):515–20.

12. Maqbool A, Schall JJ, Garcia-Espana JF, Zemel BS, Strandvik B, Stallings VA. Serum linoleic acid status as a clinical indicator of essential fatty acid status in children with cystic fibrosis. J Pediatr Gastroenterol Nutr 2008;47(5):635–44.

13. Institute of Medicine (U.S.), Panel on Macronutrients, Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. Dietary Reference Intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids. Washington (DC): National Academies Press; 2005.

14. Francis DK, Smith J, Saljuqi T, Watling RM. Oral protein calorie supplementation for children with chronic disease. Cochrane Database Syst Rev 2015;(5):CD001914.

15. Manary MJ. Local production and provision of ready-to-use therapeutic food (RUTF) spread for the treatment of severe childhood malnutrition. Food Nutr Bull 2006;27(3 Suppl):S83–9.

16. Ryan KN, Adams KP, Vosti SA, Ordiz MI, Cimo ED, Manary MJ. A comprehensive linear programming tool to optimize formulations of ready-to-use therapeutic foods: an application to Ethiopia. Am J Clin Nutr 2014;100(6):1551–8.

17. WHO. Technical note: supplementary foods for the management of moderate acute malnutrition in infants and children 6–59 months of age. Geneva: World Health Organization; 2012.

18. Jealensperger C. Technical specifications for ready to use supplementary food. Rome, Italy: World Food Program; 2016.

19. Guinard JX. Sensory and consumer testing with children. Trends Food Sci Tech 2000;11(8):273–83.

20. Weber JM, Ryan KN, Tandon R, Mathur M, Girma T, Steiner-Asiedu M, Saalia F, Zaidi S, Soo¢ § S, Okos M, et al. Acceptability of locally produced ready-to-use therapeutic foods in Ethiopia, Ghana, Pakistan and India. Matern Child Nutr 2017;13(2):x1250.

21. Nutriset. Plumpy’Nut® [Internet]. Malanouy, France: Nutriset; 2018 [cited 9 January, 2019]. Available from: https://www.nutriset.fr/products/en/plumpy-nut.

22. Abbott Laboratories. Pediasure® Grow & Gain Shake [Internet]. Chicago, IL: Abbott Laboratories; 2018 [cited 28 September, 2018]. Available from: https://abbottnutrition.com/pediasure-grow-and-gain-shake.

23. Abbott Laboratories. Ensure® Original Shake [Internet]. Chicago, IL: Abbott Laboratories; 2018 [cited 28 September, 2018]. Available from: https://abbottnutrition.com/ensure-original-shake.

24. Nestlé Health Science. Boost® Original (Retail) [Internet]. Bridgewater, NJ: Nestlé Health Science; 2017 [cited 28 September, 2018]. Available from: https://www.nestlehealthscience.us/brands/boost/boost-original-retail-hcp.

25. Nutricia. Scandishake Mix [Internet]. Dublin, Ireland: Nutricia; 2018 [cited 28 September, 2018]. Available from: http://www.nutricia.ie/products/view/scandishake_mix.

26. Groeleau V, Schall JJ, Dougherty KA, Latham NE, Maqbool A, Mascarenhas MR, Stallings VA. Effect of a dietary intervention on growth and energy expenditure in children with cystic fibrosis. J Cyst Fibros 2014;13(5):572–8.

27. Poland AL, Marcus MS, Farrell PM, Sondel SA, Kosck RE, Mischler EH. Oral supplementation with a high-fat, high-energy product improves nutritional status and alters serum lipids in patients with cystic fibrosis. J Am Diet Assoc 1995;95(4):454–9.

28. Calvo-Lerma J, Hulst JM, Asseiceira I, Claes I, Garriga M, Colombo C, Fornes V, Woodcock S, Martins T, Boon M, et 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 2017;16(4):510–18.

29. Smyth RL, Rayner O. Oral calorie supplements for cystic fibrosis. Cochrane Database Syst Rev 2017;(5):CD000406.