

## SMALL INTESTINE BACTERIAL OVERGROWTH AND FAT DIGESTION AND ABSORPTION IN CYSTIC FIBROSIS PATIENTS

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**Background.** Available data suggests that small intestine bacterial overgrowth (SIBO) may frequently occur in cystic fibrosis (CF) subjects. SIBO may result in synthesis of enterotoxic and unabsorbable metabolites which may cause mucosal damage and – additionally – interfere with digestion and absorption. Such a relationship was documented in CF mouse model. Therefore, in the present study we aimed to assess the influence of bacterial overgrowth in small intestine in CF patients on lipid digestion and absorption.

**Material and methods.** The study comprised 60 pancreatic insufficient CF patients, 30 children and 30 adults. All enrolled CF subjects were tested for the presence of SIBO using hydrogen/methane breath test with glucose loading. According to the obtained results CF patients were divided into SIBO positive and negative subgroups. Subsequently, <sup>13</sup>C-labelled mixed triglyceride breath test was performed to assess lipid digestion and absorption. Cumulative percentage dose recovery (cPDR) was considered to reflect digestion and absorption of lipids.

**Results.** SIBO was detected in 12 (40.0%) children and 11 (36.7%) adults with CF. The cPDR did not differ between SIBO positive and negative subgroups, neither when assessed separately for children (mean  $\pm$ SEM: 5.5  $\pm$ 0.8 vs. 7.4  $\pm$ 1.0%) and adults (4.9  $\pm$ 0.8 vs. 7.1  $\pm$ 0.7%) nor for the entire studied population.

**Conclusions.** Small intestine bacterial overgrowth does not seem to play a key role in lipid digestion and absorption in cystic fibrosis patients.

**Key words:** cystic fibrosis, small intestine bacterial overgrowth, lipid digestion and absorption, breath test

## INTRODUCTION

Small intestine bacterial overgrowth (SIBO) is defined as an excessive bacterial colonization of the small intestine. Gastrointestinal surgery, post-operative/ inflammatory strictures and adhesions, blind loops (surgical intestinal bypasses), villous atrophy/damage and exocrine pancreatic insufficiency were documented to be predisposing factors [Armbrecht et al. 1985, Bouhnik et al. 1999, Di Stefano et al. 2005, Singh and Toskes 2003]. Pancreatic insufficiency is a typical symptom of cystic fibrosis (CF) and develops in 85-90% of patients with this clinical entity [Walkowiak and Lisowska 2005, Walkowiak et al. 2008]. First CF symptoms usually appear in early infancy. In 10-15% neonates meconium ileus is the first manifestation, resulting from the accumulation of poorly hydrated, viscous intestinal content (due to the defect of chloride efflux and secondary passage of water) in ileocaecal region. In older subjects, both children and adults, meconium ileus equivalents may appear in the described-above mechanism [Houwen et al. 2010]. Conditions determined by the presence of significant amount of viscous, dehydrated content predispose to bacterial growth and in consequence to SIBO. Fridge et al. documented that the use of azithromycin is an additional risk factor for CF patients. On the other hand, laxatives and inhaled ipratropium were associated with a decreased risk of SIBO [Fridge et al. 2007].

Typical symptoms of SIBO comprise abdominal discomfort, bloating, flatulence, gases and appear from several minutes to hours after meal [Di Stefano et al. 2005, Fridge et al. 2007]. Due to their convergence with typical gastrointestinal manifestation in CF they may remain unnoticed. Available data suggests that SIBO occurs even in up to 30-50% of CF subjects [Houwen et al. 2010, Husebye 2005]. SIBO may result in synthesis of enterotoxic and unabsorbable metabolites which may cause mucosal damage and – additionally – interfere with digestion and absorption [Borowitz et al. 2005, Gregg 2002]. Therefore, increased energy imbalance and more severe malnutrition could be expected. Such a relationship has been documented in CF mouse model [Norkina et al. 2004].

The aim of the study was to determine the influence of bacterial overgrowth in small intestine in CF patients on lipid digestion and absorption.

## MATERIAL AND METHODS

The study comprised 60 CF patients (29 females and 31 males; Table 1). Diagnosis of CF was based on history, clinical manifestation and increased sweat chloride concentrations and confirmed by the *CFTR* gene analysis. The genotypes of the studied patients were as follows: F508del/F508del (n = 34), F508del/CFTRdel2,3 (21 kB) (n = 4), F508del/1717-1G-A (n = 2), F508del/R553X (n = 2), F508del/N1303K (n = 2), F508del/W1282X (n = 2), F508del/2143delT (n = 2), F508del/2183AA-G (n = 1), 1717-1G-A/N1303K (n = 1), F508del/unknown mutation (n = 10).

Inclusion criteria for subjects comprised the willingness to participate in the study and exocrine pancreatic insufficiency (fecal elastase – 1 concentration <100 µg/g and the presence of steatorrhea) [Walkowiak et al. 2005]. Exclusion criteria for CF comprised: antibiotic therapy (i.v. or p.os) six weeks prior to the test, liver cirrhosis, diabetes mellitus, oxygen dependency and the use of systemic corticosteroids.

Table 1. Basic epidemiological and clinical data of cystic fibrosis (CF) children (n = 30) and adults (n = 30)

Parameter	Children		Adults	
	range	mean $\pm$ SEM (Median)	range	mean $\pm$ SEM (Median)
Age, years	5-18	9.9 $\pm$ 0.6 (9.7)	19-32	25.9 $\pm$ 0.7 (25.0)
Body weight, Z-score	-2.53-1.25	-0.79 $\pm$ 0.15 (-0.82)	-2.77-1.12	-0.85 $\pm$ 0.14 (-0.87)
FEV1, %	68-121	88 $\pm$ 4 (87)	55-105	77 $\pm$ 4 (76)
Fecal elastase-1, $\mu$ g/g	0*-88	12 $\pm$ 7 (7**)	0*-75	11 $\pm$ 6 (6**)
AIAT, U/l	8-52	24.3 $\pm$ 2.1 (22)	9-56	25.2 $\pm$ 2.2 (23)
GGTP, U/l	6-185	22.9 $\pm$ 5.8 (13)	5-132	20.8 $\pm$ 5.2 (13)

All enrolled CF subjects were tested for the presence of SIBO using hydrogen/methane breath test (BT) with glucose loading. According to the obtained results CF patients were divided into SIBO positive and negative subgroups. Subsequently,  $^{13}\text{C}$ -labelled mixed triglyceride breath test ( $^{13}\text{C}$  MTG-BT) was performed to assess lipid digestion and absorption.

Glucose BT was performed after overnight fast. Patients were instructed not to eat or drink at least 12 hours before the test and to avoid slowly digesting foods like beans and similar vegetables, brans or high-fiber cereals the day before the test. Patient were not allowed to smoke, sleep or exercise vigorously for at last 1 hour before or at any time during the test. Every patient received glucose dissolved in water orally in a dose 1.5 g/kg up to the maximum total dose of 75 g. Breath samples were collected at baseline (fasting) and at 15, 30, 45, 60, 90, 120 min after glucose ingestion. Samples were analysed with QuinTron MicroLyser DP Plus (Quintron, USA). A positive BT was defined as fasting hydrogen  $\geq$  20 ppm or a rise of  $\geq$  12 ppm hydrogen or  $\geq$  6 ppm methane over baseline during the test.

$^{13}\text{C}$  MTG-BT was performed after overnight fast. Each of the studied subjects received 150 mg  $^{13}\text{C}$  mixed triglyceride with 0.25 g butter per kg body weight mixed on a slice of bread. Breath samples were collected at baseline (fasting) and at 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, 360 min after test meal ingestion. The samples were analysed with IRIS  $^{13}\text{C}$ -Analyser System (Wagner, Bremen, Germany). Cumulative percentage dose recovery (cPDR) was considered to reflect digestion and absorption of lipids. Values lower than 14.5% were considered to be abnormal [Herzog et al. 2008].

Values are expressed as ranges means  $\pm$ SEM and medians. The level of significance was set at  $p < 0.05$ . The differences in cPDR between subgroups were determined with the use of Mann-Whitney's U test.

### Ethical considerations

The protocol of the investigation was approved by Ethical Committee of Poznań University of Medical Sciences, Poland.

## RESULTS

The results of  $^{13}\text{C}$  MTG-BT were abnormal in all but one pediatric and in all but one adult subjects. SIBO was detected in 12 (40.0%) children and 11 (36.7%) adults with CF. The cPDR did not differ between SIBO positive and negative subgroups (Table 2), neither when assessed separately for children and adults nor for the entire studied population.

Table 2. Lipid digestion and absorption in cystic fibrosis (CF) children and adults based upon cumulative  $^{13}\text{C}$  dose recovery (CPDR)

Subjects (n)	CPDR		
	range	mean $\pm$ SEM (Median)	abnormal results %
Children			
CF (30)	0-20.2	6.6 $\pm$ 1.0 (5.6)	29 (96.7)
SIBO positive (12)	0-12.8	5.5 $\pm$ 0.8 (4.6)	12 (100)
SIBO negative (18)	0-20.2	7.4 $\pm$ 1.0 (6.4)	18 (94.4)
Adults			
CF (30)	0-15.6	6.2 $\pm$ 0.8 (6.2)	29 (96.7)
SIBO positive (11)	0-13.9	4.9 $\pm$ 0.8 (3.4)	11 (100)
SIBO negative (19)	0-15.6	7.1 $\pm$ 0.7 (7.2)	19 (94.7)

## DISCUSSION

There are only few data related to the occurrence of SIBO in CF subjects. According to published evidence it appears in up to 30-50% patients [Fridge et al. 2007, Lisowska et al. 2010, 2009]. Without any doubt, the influence of CF on gastrointestinal function and morphology is crucial. In the majority of CF patients several factors predisposing to SIBO coexist, in case of gastrointestinal surgery (eg. meconium ileus) the risk seems to be significantly increasing. The use of proton pump inhibitors or  $\text{H}_2$ -blockers, which are frequently prescribed in CF, may predispose to excessive bacterial colonization of small intestine [Husebye 2005]. Due to the lack of this natural barrier the overgrowth of microflora typical for airways may occur. On the other hand, the abnormal motility (eg. meconium ileus equivalents), may cause retrograde passage of colonic flora [Goldin and Wengrower 1990]. There are also other risk factors: exocrine pancreatic insufficiency, mucosal damage or atrophy, adhesions, post-inflammatory narrowing of the intestinal lumen, abnormal accumulation of surface mucus in the poorly hydrated and acidic lumen and altered biophysical and biochemical properties of intestinal mucins with potentially altered protective functions [Borowitz et al. 2005, Eggermont 1996, Singh and Toskes 2003].

Hydrogen BT is commonly accepted as a diagnostic procedure for the detection of SIBO. Although it is the best indirect method, its sensitivity and specificity is limited [Corazza et al. 1990, Kerlin and Wong 1988]. Schneider et al. [2009] questioned diagnostic value of hydrogen production in CF. Interestingly, the Authors did not find any

subject with the pathological peak of its production. Jejunal aspirate culture is considered to be a gold standard for the diagnosis of SIBO. However, this method is aggressive and not standardized. The normal values are not well established either. The combined measurement of hydrogen and methane seems to be an advantage of our study [Lisowska et al. 2009].

The CFTR null mouse model has a severe intestinal phenotype that serves as a model for meconium ileus and its equivalents. However, it has been also proved that CF mouse intestine exhibits an inflammatory status with up-regulation of components of the innate immune components. The up-regulated genes included markers for mast cells and neutrophils and acute phase proteins (serum amyloid A and complement factors). Intestinal immune cells infiltration was confirmed histologically by staining for mast cells and neutrophils [Norkina et al. 2004]. Subsequently, the authors hypothesized bacterial overgrowth as a possible cause. Quantitative PCR of bacterial 16S genomic DNA in the CF mouse small intestine revealed its 40-fold increase as compared to controls. DNA sequencing and Gram staining documented that the majority of bacteria were Gram negative, and that they colonized accumulated mucus. Antibiotic treatment (ciprofloxacin and metronidazole) for 3 weeks reduced small intestine bacterial load over 400-fold. Applied therapy decreased the expression of the inflammation-related genes to the levels similar to control levels (wild type mice). Interestingly, significant weight gain was observed.

Norkina et al. [2004 a, b] findings suggest that SIBO plays a role in intestinal inflammation and contributes to growth deficits in CF mice. Potentially, such a relationship could exist in humans. SIBO can cause inflammation and damage to intestinal mucosa and may result in diarrhea, weight loss and macrocytic anemia. However, there are no data assessing the influence of bacterial overgrowth in small intestine on clinical status of CF patients. Therefore, in the present study we defined two CF subgroups, both in children and adults, with and without SIBO and assessed lipid digestion and absorption. The lack of significant differences in cPDR suggests that such strong correlation as observed in CF mice does not exist in humans. Of course, the limited sensitivity and specificity of hydrogen-methane breath test, although higher than that of hydrogen breath test, is a drawback of the study. Further studies are needed to confirm the role of SIBO in intestinal morphology and function in CF. In conclusion, it seems that SIBO does not significantly influence lipid digestion and absorption in pancreatic insufficient CF patients.

## CONCLUSIONS

Small intestine bacterial overgrowth does not seem to play a key role in lipid digestion and absorption in cystic fibrosis patients.

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## REFERENCES

- Ambrecht U., Bosaeus I., Gillberg R., Seeberg S., Stockbruegger R., 1985. Hydrogen (H<sub>2</sub>) breath test and gastric bacteria in acid-secreting subjects and in achlorhydric and postgastrectomy patients before and after antimicrobial treatment. *Scand. J. Gastroenterol.* 20, 805-813.
- Borowitz D., Durie P.R., Clarke L.L., Werlin S.L., Taylor C.J., Semler J., De Lisle R.C., Lewindon P., Lichtman S.M., Sinaasappel M., Baker R.D., Baker S.S., Verkade H.J., Lowe M.E., Stallings V.A., Inghorbani M., Butler R., Heubi J., 2005. Gastrointestinal outcomes and confounders in cystic fibrosis. *J. Pediatr. Gastroenterol. Nutr.* 41, 273-285.
- Bouhnik Y., Alain S., Attar A., Flourié B., Raskine L., Sanson-Le Pors M.J., Rambaud J.C., 1999. Bacterial populations contaminating the upper gut in patients with small intestinal bacterial overgrowth syndrome. *Am. J. Gastroenterol.* 94, 1327-1331.
- Corazza G., Sorge M., Stocchi A., Gasparrini G., 1990. Glucose-H<sub>2</sub> breath test for small intestine bacterial overgrowth. *Gastroenterology* 98, 253-254.
- Di Stefano M., Miceli E., Missanelli A., Mazzocchi S., Corazza G.R., 2005. Absorbable vs. non-absorbable antibiotics in the treatment of small intestine bacterial overgrowth in patients with blind-loop syndrome. *Aliment. Pharmacol. Ther.* 21, 985-992.
- Di Stefano M., Miceli E., Missanelli A., Corazza G.R., 2005. Treatment of small intestine bacterial overgrowth. *Eur. Rev. Med. Pharmacol. Sci.* 9, 217-222.
- Eggermont E., 1996. Gastrointestinal manifestation in cystic fibrosis. *Eur. J. Gastroenterol. Hepatol.* 8, 731-738.
- Fridge J.L., Conrad C., Gerson L., Casteillo R.O., Cox K., 2007. Risk factors for small bowel bacterial overgrowth in cystic fibrosis. *J. Pediatr. Gastroenterol. Nutr.* 44, 2, 212-217.
- Goldin E., Wengrower D., 1990. Diarrhea in hypothyroidism: bacterial overgrowth as a possible etiology. *J. Clin. Gastroenterol.* 12, 98-99.
- Gregg C.R., 2002. Enteric bacterial flora and bacterial overgrowth syndrome. *Semin. Gastrointest. Dis.* 13, 4, 200-209.
- Herzog D.C., Delvin E.E., Albert C., Marcotte J.E., Pelletier V.A., Seidman E.G., 2008. <sup>13</sup>C-labeled mixed triglyceride breath test (<sup>13</sup>C MTG-BT) in healthy children and children with cystic fibrosis (CF) under pancreatic enzyme replacement therapy (PERT): A pilot study. *Clin. Biochem.* 41, 1489-1492.
- Houwen R.H., van der Doef H.P., Sermet I., Munck A., Hauser B., Walkowiak J., Robberecht E., Colombo C., Sinaasappel M., Wilschanski M., on behalf of the ESPGHAN Cystic Fibrosis Working Group, 2010. Defining DIOS and constipation in cystic fibrosis with a multicentre study in the incidence, characteristics, and treatment of DIOS. *J. Pediatr. Gastroenterol. Nutr.* 1, 50, 38-42.
- Husebye E., 2005. The pathogenesis of gastrointestinal bacterial overgrowth. *Chemotherapy* 51 (Suppl. 1), 1-22.
- Kerlin P., Wong L., 1988. Breath hydrogen testing in bacterial overgrowth of the small intestine. *Gastroenterology* 95, 982-988.
- Lisowska A., Mądry E., Pogorzelski A., Szydłowski J., Radzikowski A., Walkowiak J., 2010. Small intestine bacterial overgrowth does not correspond to intestinal inflammation in cystic fibrosis. *Scand. J. Clin. Lab. Invest.* Jun 21 [Epub ahead of print].
- Lisowska A., Wójtowicz J., Walkowiak J., 2009. Small intestine bacterial overgrowth is frequent in cystic fibrosis: combined hydrogen and methane measurements are required for its detection. *Acta Biochim. Pol.* 56, 4, 631-634.
- Norkina O., Burnett D.G., De Lisle R.C., 2004 a. Bacterial overgrowth in cystic fibrosis transmembrane conductance regulator null mouse small intestine. *Infect. Immun.* 72, 6040-6049.
- Norkina O., Kaur S., Ziemer D., De Lisle R.C., 2004 b. Inflammation of the cystic fibrosis mouse small intestine. *Am. J. Physiol. Gastrointest. Liver. Physiol.* 286, G1032-1041.
- Schneider A.R.J., Klueber S., Posselt H.-G., Funk B., Murzynski L., Caspary W.F., Stein J., 2009. Application of the glucose hydrogen breath test for the detection of bacterial overgrowth in patients with cystic fibrosis – a reliable method? *Dig. Dis. Sci.* 54, 1730-1735.

- Singh V.V., Toskes P.P., 2003. Small bowel bacterial overgrowth: presentation, diagnosis and treatment. *Curr. Gastroenterol. Rep.* 5, 365-372.
- Walkowiak J., Lisowska A., 2005. Pancreatic enzyme therapy and gastrointestinal symptoms in patients with cystic fibrosis. *J. Pediatr.* 147, 6, 870-871.
- Walkowiak J., Lisowska A., Błaszczyński M., 2008. The changing face of the exocrine pancreas in cystic fibrosis: pancreatic sufficiency, pancreatitis and genotype. *Eur. J. Gastroenterol. Hepatol.* 20, 3, 157-160.
- Walkowiak J., Nousia-Arvanitakis S., Henker J., Stern M., Sinaasappel M., Dodge J.A., 2005. Indirect pancreatic function tests in children. *J. Pediatr. Gastroenterol. Nutr.* 40, 2, 107-114.

## JELITOWY PRZEROST BAKTERYJNY A TRAWIENIE I WCHŁANIANIE TŁUSZCZÓW U CHORYCH NA MUKOWISCYDOZĘ

**Wstęp.** W świetle dostępnych danych uważa się, że zespół jelitowego przerostu bakteryjnego (ZJPB) często występuje u chorych na mukowiscydozę (ang. *cystic fibrosis* – CF). ZJPB może powodować powstanie toksycznych i niewchłanianych metabolitów, mogących uszkadzać błonę śluzową jelita cienkiego, a także zaburzać trawienie i wchłanianie. Występowanie powyższej zależności udokumentowano w mysim modelu CF. Dlatego w przeprowadzonym badaniu podjęto próbę oceny wpływu ZJPB na trawienie i wchłanianie tłuszczów u chorych na CF.

**Materiał i metody.** Badaniem objęto 60 niewydolnych trzustkowo pacjentów z CF, w tym 30 dzieci i 30 dorosłych. U wszystkich badanych oceniono występowanie ZJPB z zastosowaniem wodorowo-metanowego testu oddechowego, przeprowadzonego po dostnym obciążeniu glukozą. Stosownie do uzyskanych wyników pacjentów podzielono na dwie podgrupy: z ZJPB i bez ZJPB. Następnie pacjenci zostali poddani testowi oddechowemu z <sup>13</sup>C-miesznymi trójglicerydami w celu oceny trawienia i wchłaniania tłuszczów. Przyjęto, iż kumulacyjny odsetek odzysku dawki stanowi odzwierciedlenie efektywności powyższego procesu.

**Wyniki.** Występowanie ZJPB stwierdzono u 12 dzieci (40%) i 11 dorosłych (36,7%) pacjentów. Kumulacyjny odsetek odzysku dawki nie różnił się istotnie w grupach z ZJPB i bez ZJPB, zarówno dzieci ( $X\bar{s} \pm SEM$ : 5,5  $\pm$  0,8 vs. 7,4  $\pm$  1,0%) oraz dorosłych ( $X\bar{s} \pm SEM$ : 4,9  $\pm$  0,8 vs. 7,1  $\pm$  0,7%), jak i całej populacji.

**Wnioski.** Zespół jelitowego przerostu bakteryjnego nie wydaje się odgrywać kluczowej roli w trawieniu i wchłanianiu tłuszczów u chorych na mukowiscydozę.

**Słowa kluczowe:** mukowiscydoza, zespół jelitowego przerostu bakteryjnego, trawienie i wchłanianie tłuszczów, test oddechowy

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