Brief Communications

Detection and interpretation of fecal host mRNA in rural Malawian infants aged 6–12 months at risk for environmental enteric dysfunction

M Isabel Ordiz[1,*], Karl Wold[1,*], Yankho Kaimila[2], Oscar Divala[2], Madeline Gilstrap[1], Henry Z Lu[1] and Mark J Manary[1,2,3]

[1]Department of Pediatrics, Washington University at Saint Louis, St. Louis, MO 63110, USA; [2]School of Public Health and Family Medicine, University of Malawi, Blantyre 3, Malawi; [3]Children's Nutrition Research Center, Baylor College of Medicine, Houston, TX 77030, USA

*These authors contributed equally to this work.

Corresponding author: Mark J Manary. Email: manary@kids.wustl.edu

Impact statement

Environmental enteric dysfunction (EED) is associated with reduced linear growth. The dual sugar absorption test has been used as a non-invasive method to determine the gut health of individuals. Alternative methods using fecal host mRNAs as predictors of the gut health are promising. In older children, we have determined that seven transcripts can predict the gut health in a random forest model. Our current study determined that the host fecal mRNA is abundant in infants and toddlers alike. Severe EED in rural Malawian children is less prevalent in infants than in young children. REG1A and CDX1 are associated with gut health. Fecal host mRNA may well be a means to assess gut health in African infants, but the panel of transcripts used to do this will differ from that in older children.

Abstract

Recent studies have suggested that environmental enteric dysfunction can be assessed in rural African children by measuring levels of fecal mRNA transcripts. The field collection of fecal samples is less invasive and cumbersome than administration of the lactulose:mannitol test, which is typically used to assess environmental enteric dysfunction. This study sought to determine if, as in children aged 12–60 months, an array of seven fecal host transcripts (CD53, CDX1, HLA-DRA, TNF, S100A8, MUC12, and REG1A) could predict environmental enteric dysfunction in rural African infants. Host fecal transcript abundance was correlated to the percentage of lactulose (%L) excreted in the urine for 340 samples from Malawian children aged 6–12 months. Permeability was categorized as not severe (%L < 0.45) and severe (%L ≥ 0.45). This study found the prevalence of severe environmental enteric dysfunction to be 114/834 (14%), lower than what was previously reported for 12–60 months old children, 595/1521 (39%, $P = 0.001$). In linear regression analysis with the seven host transcripts, two were associated with %L: β coefficients of -1.843 ($P = 0.035$) and 0.215 ($P = 0.006$) for CDX1 and REG1A, respectively. The seven fecal host transcripts in a random forest model did not predict severe environmental enteric dysfunction. Future models utilizing different transcripts identified from an untargeted, agnostic assessment of all potential host transcripts could provide accurate predictions of environmental enteric dysfunction in infants.

Keywords: Environmental enteric dysfunction, fecal biomarkers, gut health, dual sugar absorption test, droplet digital polymerase chain reaction

***Experimental Biology and Medicine* 2018; 243: 985–989. DOI: 10.1177/1535370218794418**

Introduction

Environmental enteric dysfunction (EED) is asymptomatic upper small bowel mucosal inflammation characterized by villus blunting on histologic examination.[1,2] EED is associated with stunting, which has multiple causal pathways, results in increased morbidity and mortality and its prevention is of utmost interest to the global health community.[3] Since the greatest growth faltering is seen in infants and young children, EED is thought to occur in this demographic.[4,5] EED and stunting are prevalent in sub-Saharan Africa and south Asia, where direct examination of the small bowel is rarely employed.[6] Rather, EED is

often diagnosed using a sugar permeability test, where saccharides that are not actively transported across the mucosa are given orally, a fraction of these sugars leak across the cell junctions and then are excreted and quantified in the urine. The most commonly used sugar permeability test is the lactulose:mannitol (L:M) test.[7] The L:M test is cumbersome because it requires a complete urine collection over 2–5 h, and the test perturbs small bowel permeability, therefore it cannot reliably be repeated without waiting several days.

The validity of L:M test has recently been questioned on the basis of cell junction architecture and inconsistencies between empiric observations and the premises of the L:M test.[8] It has been recommended that total urinary lactulose (L) excretion be used instead of the L:M test, although this does not address the practical constraints that sugar absorption testing imposes.

A recent alternative to sugar absorption testing for EED is quantification of a panel of host mRNA from feces.[9–11] Our lab has extensively studied this method in rural African children aged two to five years old, and found that EED can be predicted using a panel of four mRNAs: CD53, MUC12, HLA-DRA, and TNF.[11] Since EED is thought to be more consequential when it occurs in infants, it is of interest whether fecal mRNA can be detected in this population.[11–13] This study tested the hypotheses that host mRNAs would be detectable in similar quantities in fecal samples from infants as they are in young children, and that a similar panel of mRNAs would predict EED in infants as does in children.

Materials and methods

Subjects

Subjects resided in rural southern Malawi, were aged 6 to12 months, and participated in a clinical trial of legume supplementation in which %L was measured simultaneously with a fecal collection.[12] Infants with acute malnutrition, chronic debilitating illness, congenital deformity, or recent diarrhea were excluded. Families relied on subsistence farming, retrieved their water from boreholes or wells, resided in unelectrified mud huts, and were at high risk for EED. Ethical approval for this study was obtained from the University of Malawi, College of Medicine and Washington University.

Study design

This was an observational study to determine (1) whether similar amounts of mRNA can be isolated and detected in infants as has been quantified in older children, (2) the extent to which selected fecal host mRNAs predict %L in infants.

Participation

Participation has been described in detail previously.[12] Briefly, during a clinic visit at approximately 6, 9 and 12 months of age, information regarding the demographics, dietary intake, and household sanitation practices was collected from the child's primary caretaker. Length, weight, and mid-upper arm circumference (MUAC) were measured.

All of the subjects underwent a carefully conducted L:M test with adequate urine collection and sugar excretion.[13] Children consumed L (5 g) and mannitol (1 g) dissolved in 20 mL of water. Once the solution was ingested, an adhesive urine bag was attached to the child's perineum and monitored for urine output. When urine was noted in the bag, the urine transferred into a clean container, contained 10 mg of merthiolate. Immediately after removing a urine bag, a new bag was attached. Two hours after sugar ingestion, children were encouraged to drink water to facilitate urination and 3 h after sugar ingestion, children were allowed to eat. Once the child voided for the first time after 4 h, the L:M test was finished. The volume of the collected urine was measured. An aliquot of the child's urine was added to a cryovial and flash frozen in liquid nitrogen. These samples were moved to a −20°C freezer until they were transported at −70°C to Washington University. The concentrations of L and M were analyzed by HPLC. The designation of severe EED was set at $\%L \geq 0.45$, because it was found to be associated with growth faltering in older Malawian children (12–61 months).[13–15]

Fresh stool samples were collected using a small, clean, non-absorbent plastic diaper. Immediately upon defecation, the stool was mixed with a metal spatula, transferred to a cryovial, and flash frozen in liquid nitrogen without buffers, enzymes, or preservative solutions. Samples were transferred to a −80°C freezer and transported to Washington University at −70°C, where they were processed and analyzed for fecal mRNA.

Isolation and detection of fecal RNA

Fecal nucleic acid extractions were prepared using NucliSENS® easyMAG® system (bioMerieux, Durham, NC) and a modified version of the protocol of Agapova *et al.*[9] and Stauber *et al.*[16] with 200–300 mg of frozen stool. Quantitative PCR assays were performed using duplexed FAM and VIC TaqMan assays in a droplet digital PCR system (QX200; Bio-Rad Laboratories, Inc., Hercules, CA) for the following seven targets: CD53, CDX1, HLA-DRA, MUC12, REG1A, S100A8, and TNF. All of the probes used were designed specifically around the exon boundaries to eliminate cross reactivity with microbial DNA or RNA and with genomic DNA. Duplicate reactions were prepared. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) assays were performed for each sample for normalization of all targets. PCR reactions were dispersed into droplets using the QX200 droplet generator and transferred to a 96-well PCR plate. End point PCR was performed in a C1000 Touch thermal cycler with the following conditions: 50°C 30′, 95°C 10′, 40 cycles of 94°C 30″ followed by 60°C 1′, 98°C 10′ and cooling to 4°C. The fluorescence of each droplet was quantified in the droplet reader.

Absolute quantification was performed using QuantaSoft software (BioRad, Santa Clara, CA). Thresholds were set to distinguish between positive and negative droplets. Samples with insufficient negative

droplets for a clear baseline were excluded from analysis. Output from QuantaSoft included concentration and droplet counts for each target. The average concentration for duplicate wells was then calculated and normalized to GAPDH. Results were reported as Target/GAPDH ratios.

Data analyses

Children were divided into two groups, those with severe EED having %L $\geq$ 0.45, and those with no EED or moderate EED having %L $<$ 0.45. The group characteristics were compared using Student's *t*-test for continuous parameters and Fisher's exact test for categorical parameters. The ddPCR target measurements were compared to those found in our previously published work, to determine copy numbers typically being detected.[16] Spearman's correlation coefficients were calculated to explore the relationship with the transcripts analyzed. Host mRNAs from children with severe EED were compared to those with no or moderate EED after log transformation of the data using regression modeling to control for the correlation between transcript copy numbers.

Random forest modeling was conducted as previously described for determination of a binary categorical outcome, no EED or moderate EED versus severe EED. Random forest modeling was performed using the rf package in R (www.r-project.org, Vienna, Austria). Cross validation of models was done by removing 30 children from the data set, and testing the accuracy of the model in this naïve group.

Table 1. Characteristics of Malawian children at risk for environmental enteric enteropathy.

Characteristic	No or moderate EED *n* = 281	Severe EED *n* = 59
Sex, male	150 (53%)	37 (63%)
Siblings	2.6 ± 1.9	2.5 ± 1.9
Mother alive	280 (100%)	59 (100%)
Improve roofing material on home (metal sheets)	65 (23%)	8 (14%)
Radio in home	94 (33%)	19 (32%)
Bicycle in home	142 (51%)	22 (37%)
Animals sleep with children in home	86 (31%)	17 (29%)
Has a clean source of water	192 (68%)	40 (68%)
Home has a pit latrine	44 (16%)	10 (17%)
Breastfed at the time of the study	281 (100%)	59 (100%)
Age, mo.	7.1 ± 1.7	7.5 ± 1.9
Length-for-age, z score	−1.2 ± 1.0	−1.2 ± 1.1
Weight-for-height, z score	0.24 ± 0.88	0.02 ± 0.82
Mid-upper arm circumference, cm	14.1 ± 0.9	13.9 ± 0.9

Note: Values expressed as mean ± SD or no (%). Differences for all of the characteristics were not significant using Student's *t*-test for continuous parameters and Fisher's exact test for categorical parameters.
EED: environmental enteric dysfunction.

Results

A total of 340 pairs of %L measurements and fecal samples were assessed. Children with %L $\geq$ 0.45 were indistinguishable from those with %L $<$ 0.45 (Table 1).

A total of 3586 ddPCR measurements were run in duplicate, with a median coefficient of variation of 3.5% and a mean CV of 6.7%. All seven targets were detected in more than 99% of samples in quantities similar to older rural Malawian children (Table 2).[16] For the 6–12 months old children, 114/834 (14%) had %L $\geq$ 0.45, while among older children, aged 12–36 months, the fraction with %L $\geq$0.45 was 595/1521 (39%) ($P = 0.001$).[17]

The seven transcripts measured were segregated into three groups based on correlation with each other: (1) CDX1, HLA-DRA, MUC12, (2) CD53, S100A8, TNF, and (3) REG1A (Table 3). Comparison of the copy number of each of the seven transcripts for infants with %L $\geq$ 0.45 with those with %L $<$ 0.45 revealed that only CDX1 differed between these two groups (Table 4).

Regression modeling found two transcripts to be significant predictors of severe EED, CDX1, and REG1A, which had β coefficients of −1.843 ($P = 0.035$) and 0.215 ($P = 0.006$), respectively.

Random forest modeling with these seven transcripts and the characteristics of age, sex, clean water used in the home, presence of a pit latrine in the home, animals sleep in same room as the child, WHZ, and HAZ was undertaken. The most specific and sensitive random forest model to detect children with severe EED included just five of these independent variables: CD53, REG1A, animals sleep in the same room as the child, WHZ, and LAZ. The random forest model was 29% sensitive and 88% specific ($n = 310$, node size = 4, max node = 10, mtry = 3). Validation of

Table 2. Mean and median transcript abundance in young (<12 month) and older (12–61 months) Malawian children.[a]

	Children <12 month *n* = 340		Children 12–61 months	
mRNA transcript	Mean ± SD	Median (25th, 75th %tiles)	Mean ± SD (*n*)	Median (25th, 75th %tiles)
CD53	0.121 ± 0.170	0.062 (0.026, 0.159)	0.076 ± 0.124 (324)	0.035 (0.013, 0.091)
CDX1	0.024 ± 0.018	0.018 (0.012, 0.029)	0.047 ± 0.346 (562)	0.027 (0.016, 0.042)
HLA-DRA	0.078 ± 0.069	0.060 (0.031, 0.100)	0.220 ± 0.170 (567)	0.174 (0.103, 0.281)
MUC12	0.351 ± 0.384	0.217 (0.121, 0.450)	0.447 ± 0.499 (319)	0.294 (0.163, 0.539)
REG1A	0.100 ± 0.151	0.041 (0.016, 0.101)	0.114 ± 0.268 (622)	0.042 (0.018, 0.107)
S100A8	1.927 ± 2.488	1.406 (0.472, 2.304)	0.979 ± 1.738 (550)	0.386 (0.154, 1.169)
TNF	0.016 ± 0.018	0.010 (0.005, 0.020)	0.008 ± 0.015 (571)	0.004 (0.002, 0.008)

[a]The unit of measure used for each transcript was copies/copy of GAPDH. Comparisons were not made between the transcript abundance in older and younger children, because these populations are remarkably disparate from each other in terms of diet and environmental exposures.

Table 3. Spearman correlation coefficients between measured transcript values.[a]

CDX1	□					
HLA-DRA	0.144 (0.02)	□				
MUC12	0.579 (<0.001)	0.192 (<0.001)	□			
CD53	−0.177 (0.001)	0.428 (<0.001)	−0.1 (0.07)	□		
S100A8	−0.227 (<0.001)	0.254 (<0.001)	−0.159 (0.006)	0.800 (<0.001)	□	
TNF	0.061 (0.21)	0.416 (<0.001)	0.134 (0.03)	0.736 (<0.001)	0.639 (<0.001)	□
REG1A	0.244 (<0.001)	0.215 (<0.001)	0.003 (0.61)	0.049 (0.23)	0.064 (0.25)	0.142 (0.02)
	CDX1	HLA_DRA	MUC12	CD53	S100A8	TNF

[a]The unit of measure used for each transcript was copies/copy of GAPDH. *P* values noted in ( ) are below the coefficient. Based on correlation coefficients, three clusters of transcripts were identified denoted by the dashed lines in the first column: (1) CDX1, HLA-DRA and MUC 12, (2) CD53, S100A8, and TNF, (3) REG1A.

Table 4. Mean and median transcript levels in severe and no or moderate EED Malawian infants.

	Children severe EED $n = 59$		Children no or moderate EED $n = 281$	
mRNA transcript	Mean ± SD	Median (25th, 75th %tiles)	Mean ± SD	Median (25th, 75th %tiles)
CD53	0.112 ± 0.119	0.059 (0.024, 0.203)	0.127 ± 0.277	0.055 (0.025, 0.133)
CDX1[a]	0.020 ± 0.014	0.018 (0.010, 0.025)	0.027 ± 0.027	0.019 (0.013, 0.031)
HLA-DRA	0.096 ± 0.158	0.064 (0.039, 0.102)	0.088 ± 0.198	0.061 (0.031, 0.098)
MUC12	0.306 ± 0.268	0.192 (0.121, 0.393)	0.395 ± 0.531	0.217 (0.123, 0.473)
REG1A	0.182 ± 0.259	0.046 (0.016, 0.283)	0.142 ± 0.387	0.047 (0.016, 0.114)
S100A8	2.09 ± 2.51	1.33 (0.38, 2.36)	2.68 ± 7.01	1.10 (0.50, 2.55)
TNF	0.019± 0.029	0.009 (0.005, 0.024)	0.030 ± 0.109	0.010 (0.005, 0.021)

[a]Children with severe EED different from those without severe EED when compared by Student's *t*-test after log transformation.

model with 30 samples removed from the model creation exercises yielded 31% sensitivity and 85% specificity.

Discussion

The findings of this study suggest that severe EED, defined as $\%L \geq 0.45$, is less prevalent in Malawian infants aged 6–12 months than what has been seen in children aged 1–5 years from the same population.[16] ddPCR assessment of feces from the study population reproducibly detected CD53, CDX1, HLA-DRA, MUC12, REG1A, S100A8, and TNF in quantities similar to those found in older children; the mean values were within three fold of each other. CDX1 was associated with $\%L < 0.45$ and REG1A was associated with $\%L \geq 0.45$. A random forest model to predict the presence of severe EED with 80% sensitivity and specificity in the study population was not found using these transcripts.

The subjects in this study were the children of rural subsistence farmers in southern Malawi. Therefore, the conclusions drawn from this work may not be applicable to children from urban contexts, or from other geographic areas around the globe.

The origin and detection of gut transcripts were recently elegantly studied in a mouse model of small intestinal injury.[18] The cells shed into the gut lumen, referred to as the exfoliome, represented cells from both the colon and small intestine, with about 80% of the genes identified present in both tissues, thus one cannot be sure of the site of origin of the transcript. However, sparse canonical correlational analysis showed the exfoliome was better able to differentiate transcripts from damaged small intestine than from the colon. This work indicates that the exfoliome can be a non-invasive assessment tool for the inflamed small intestine. The RNA integrity of the exfoliome was lower than that seen in tissue samples. Certainly the RNA which we detected in the Malawian children is largely derived from the exfoliome. This animal model supports our assumption that a unique transcriptomic signature may be found for EED.

It is interesting to note that severe EED was less prevalent in the infants than in the older children. This finding is contrary to what many experts might speculate, based on the timing of growth faltering. Older and younger children lived in the same setting, received the same dose of L and the L:M test was conducted in an identical manner. The average length of the small bowel in the younger age group is estimated to be 75% that of the older group.[19] The finding that severe EED is less prevalent in younger children is unlikely to be due to differences in bowel length, but probably genuinely reflects differences in mucosal cell junction architecture between the two groups. Less permeability among infants might be the consequence of optimal breast feeding, as breast milk has many functional components that nurture gut health. It might also be that the small bowel is more chronically irritated in the older children, as they have more intimate exposure to environmental

contaminants. These speculations as to why this intriguing difference exists would need further study to better understand its associations and consequences.

Though this study was unable to develop a model to predict EED in Malawian infants, future studies that incorporate different mRNA transcripts into the analysis could yield useful models. The seven transcripts used as targets here were selected because they have previously been shown to predict the presence of EED in older children from the very same setting. By using an untargeted, agnostic, whole-transcriptome analysis, as in Yu *et al.*,[10] it may well be possible to develop a predictive panel of stool RNA biomarkers that can serve as predictors of EED in young children.

A fecal biomarker that does not perturb the small bowel integrity when tested remains a need to better elucidate the role of EED in infant growth faltering in rural Africa, and a panel of host mRNAs holds promise for this purpose.

Authors' contributions: MJM and MIO designed the study; KW, YK and OD collected samples, MIO, HZL, YK, OD and MG analyzed the samples; KW, MIO, OD and MJM analyzed the data. KW and MJM wrote the first draft of the manuscript. All authors have seen the final version of the manuscript and approve of its contents.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

FUNDING

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the United States Agency for International Development (USAID), as part of Feed the Future, the U.S. Government's global hunger and food security initiative, under the terms of Cooperative Agreement No. EDH-A-00-07-00005-00, and the Children's Discovery Institute of Washington University and St Louis Children's Hospital. The opinions expressed herein are those of the authors and do not necessarily reflect the views of USAID or the U.S. government.

REFERENCES

1. Syed S, Ali A, Duggan C. Environmental enteric dysfunction in children: a review. *J Pediatr Gastroenterol Nutr* 2016;**63**:6–14
2. Campbell DI, Elia M, Lunn PG. Growth faltering in rural Gambian infants is associated with impaired small intestinal barrier function, leading to endotoxemia and systemic inflammation. *J Nutr* 2003;**133**:1332–8
3. Black RE, Allen LH, Bhutta ZA, Caulfield LE, De Onis M, Ezzati M, Mathers C, Rivera J; Maternal and child undernutrition study group. Maternal and child undernutrition: global and regional exposures and health consequences. *Lancet* 2008;**371**:243–60
4. Victora CG, de Onis M, Hallal PC, Blössner M, Shrimpton R. Worldwide timing of growth faltering: revisiting implications for interventions. *Pediatrics* 2010;**125**:e473–80
5. Campbell DI, Lunn PG, Elia M. Age-related association of small intestinal mucosal enteropathy with nutritional status in rural Gambian children. *Br J Nutr* 2002;**88**:499–505
6. Prendergast A, Kelly P. Enteropathies in the developing world: neglected effects on global health. *Am J Trop Med Hyg* 2012;**86**:756–63
7. Denno DM, VanBuskirk K, Nelson ZC, Musser CA, Hay Burgess DC, Tarr PI. Use of the lactulose to mannitol ratio to evaluate childhood environmental enteric dysfunction: a systematic review. *Clin Infect Dis* 2014;**59**:S213–9
8. Ordiz MI, Davitt C, Stephenson K, Agapova S, Divala O, Shaikh N, Manary MJ. Interpretation of the lactulose:mannitol test in rural Malawian children at risk for perturbations in intestinal permeability. *Exp Biol Med* 2018;**243**:677–83
9. Agapova S, Stephenson K, Manary M, Weisz A, Tarr PI, Mkakosya R, Maleta K, Shulman RJ, Manary M, Shaikh N. Detection of low-concentration host mRNA transcripts in Malawian children at risk for environmental enteropathy. *J Pediatr Gastroenterol Nutr* 2013;**56**:66–71
10. Yu J, Ordiz MI, Stauber J, Shaikh N, Trehan I, Barnell E, Head RD, Maleta K, Tarr PI, Manary MJ. Environmental enteric dysfunction includes a broad spectrum of inflammatory responses and epithelial repair processes. *Cell Mol Gastroenterol Hepatol* 2016;**2**:158–74
11. Ordiz MI, Shaikh N, Trehan I, Maleta K, Stauber J, Shulman R, Devaraj S, Tarr PI, Manary MJ. Environmental enteric dysfunction is associated with poor linear growth and can be identified by host fecal mRNAs. *J Pediatr Gastroenterol Nutr* 2016;**63**:453–9
12. Stephenson KB, Agapova SE, Divala O, Kaimila Y, Maleta KM, Thakwalakwa C, Ordiz MI, Trehan I, Manary MJ. Complementary feeding with cowpea reduces growth faltering in rural Malawian infants: a blind, randomized controlled clinical trial. *Am J Clin Nutr* 2017;**106**:1500–7
13. Trehan I, Benzoni NS, Wang AZ, Bollinger LB, Ngoma TN, Chimimba UK, Stephenson KB, Agapova SE, Maleta KM, Manary MJ. Common beans and cowpeas as complementary foods to reduce environmental enteric dysfunction and stunting in Malawian children: study protocol for two randomized controlled trials. *Trials* 2015;**16**:520
14. Weisz AJ, Manary MJ, Stephenson K, Agapova S, Manary FG, Thakwalakwa C, Shulman RJ, Manary MJ. Abnormal gut integrity is associated with reduced linear growth in rural Malawian children. *J Pediatr Gastroenterol Nutr* 2012;**55**:747–50
15. Smith HE, Ryan KN, Stephenson KB, Westcott C, Thakwalakwa C, Maleta K, Cheng JY, Brenna JT, Shulman RJ, Trehan I, Manary MJ. Multiple micronutrient supplementation transiently ameliorates environmental enteropathy in Malawian children aged 12-35 months in a randomized controlled clinical trial. *J Nutr* 2014;**144**:2059–65
16. Stauber J, Shaikh N, Ordiz MI, Tarr PI, Manary MJ. Droplet digital PCR quantifies host inflammatory transcripts in feces reliably and reproducibly. *Cell Immunol* 2016;**303**:43–9
17. Agapova S, Stephenson KB, Divala O, Kaimila Y, Maleta K, Thakwalakwa C, Ordiz MI, Trehan I, Manary M. Additional common bean in the diet of Malawian children does not affect linear growth, but reduces intestinal permeability. *J Nutr* 2018;**148**:267–74
18. Whitfield-Cargile CM, Cohen ND, He K, Ivanov I, Goldsby JS, Chamoun-Emanuelli A, Weeks BR, Davidson LA, Chapkin RS. The non-invasive exfoliated transcriptome (exfoliome) reflects the tissue level transcriptome in a mouse model of NSAID enteropathy. *Sci Rep* 2017;**7**:14687
19. Weaver LT, Austin S, Cole TJ. Small intestinal length: a factor essential for gut adaptation. *Gut* 1991;**32**:1321–3

(Received May 28, 2018, Accepted July 24, 2018)