INTESTINAL ANTIMICROBIAL GENE EXPRESSION: IMPACT OF MICRONUTRIENTS IN MALNOURISHED ADULTS DURING A RANDOMISED TRIAL

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Abstract

Background—As both micronutrients and antimicrobial peptides protect against diarrhoea, we looked for an effect on intestinal antimicrobial peptide gene expression during a randomised controlled trial of multiple micronutrient (MM) supplementation.

Methods—Consenting adults (n=287) in Lusaka, Zambia were randomised to a daily MM supplement or placebo, and followed for 3.3 years with a cross-over after 2 years. Intestinal biopsies were taken at annual intervals and mRNA of the intestinal antimicrobial peptides HD5, HD6, hBD1, hBD2 and LL-37 were quantified by real time RT-PCR. Samples were also taken during diarrhoea episodes and after convalescence.

Results—There was no effect overall of treatment allocation. However in malnourished adults (body mass index <18.5kg/m^2), HD5 mRNA was increased by 0.8 log transcripts/µg total RNA in MM recipients compared to placebo (*P*=0.007). During diarrhoea, HD5 expression was reduced by 0.8 log transcripts in placebo recipients (*P*=0.02) but not in MM recipients nor after the cross-over. Correlations between HD5 and nutritional status were found which were sex-specific but not explained by serum leptin or adiponectin concentrations.

Conclusion—Micronutrient supplementation was associated with up-regulation of HD5 only in malnourished adults. Interactions between antimicrobial gene expression and nutritional status may help explain increased infection risk in malnutrition.

Keywords

defensin; cathelicidin; antimicrobial; micronutrient; intestinal infection; innate immunity; diarrhoea; randomised controlled trial; leptin; adiponectin

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Introduction

Diarrhoeal disease remains a major cause of morbidity and mortality in children, especially malnourished and HIV-infected children, in tropical countries [1,2,3]. Despite impressive roll-out of anti-retroviral therapy in many parts of Africa, including Zambia [4], HIV infected adults and children continue to present with gastrointestinal opportunistic infections, many of which are still difficult to treat. Reducing morbidity and mortality from gastrointestinal infections presents a major challenge because significant improvements in water supply and sanitation will require economic transformation of large areas of Africa and Asia,. Understanding mucosal defence mechanisms in the intestine may lead to development of novel strategies to augment resistance to intestinal infection.

Evidence from a considerable body of randomised controlled trials indicates that micronutrient supplementation can prevent diarrhoea in children [5,6] (though not in all trials [7]) and in HIV infected adults too, though data are fewer [8,9]. There is also evidence that zinc supplementation reduces morbidity and mortality from diarrhoeal disease when started during the episode [6, 10]. However, it is not at all clear how this protective effect is mediated. Evidence of an effect on cell mediated immunity is inconclusive [11], and some promising evidence has been discredited [12], but there is some evidence that in HIV infected adults micronutrients can increase CD4 count [13]. Very little evidence, if any, exists on the impact of nutritional interventions on innate immunity and host defence mechanisms. In previous work we found that gene expression of α-defensins is lower in adults living in Misisi, Lusaka, than in London, UK [14], and we postulated that this may reflect a nutritional deficiency. In preliminary work for this trial, between 10 and 20% of adults in this population were shown to have low blood concentrations of vitamin A, folate or zinc, and 4 placebo recipients developed pellagra during the course of the trial, so this population appears to have several borderline micronutrient deficiencies.

Mucosal defence in the intestine relies on physical and chemical barriers, as well as regulated pathways of innate and adaptive immunity. A critical component of these barriers is the layer of antimicrobial molecules secreted onto the mucosa, including defensins, cathelicidin LL-37, and other cationic peptides and proteins such as lysozyme [15,16,17]. In the present study we set out to determine the extent to which antimicrobial peptide host defence mechanisms are dependent on micronutrient status, by conducting a randomised placebo-controlled trial of daily multiple micronutrient supplementation [18]. Small intestinal biopsies were collected annually in a scheduled programme over 3 years and 4 months (3.3 years) and analysed for gene expression and histologically. As there is evidence that intestinal infection suppresses expression of antimicrobial peptides [19,20] and that enterotoxins can do the same in vitro [21], we also collected biopsies during diarrhoeal episodes and after convalescence to determine if micronutrient supplementation affects antimicrobial gene expression during the dynamic challenge of active infection.

Study design and methods

The design of the randomised controlled trial during which these intestinal biopsy samples were collected has been reported in detail elsewhere, together with data on the effect of the MM supplement on diarrhoeal disease, CD4 count, and mortality [18]. Briefly, 500 adults resident in one section of Misisi compound, Lusaka, Zambia, were recruited into a randomised controlled trial of micronutrient supplementation compared to placebo and gave consent. The composition of the multiple micronutrient (MM) supplement was designed to achieve replenishment of 15 nutrients at physiological levels [18]. Ethical approval was obtained from the Research Ethics Committees of the University of Zambia and the London School of Hygiene and Tropical Medicine. The trial was registered as ISRCTN31173864.

Small intestinal biopsies were collected from the second or third part of the duodenum by an experienced endoscopist (PK) using Pentax fibre-optic FG29W gastroscopes under sedation with midazolam (usual dose 5mg). Two sets of biopsies were collected.

In the first set, participants were invited to come at random for endoscopy in a rolling programme so that biopsies were collected throughout the year (these will be referred to as 'scheduled biopsies'). These participants were subsequently invited at annual intervals so that each participant was biopsied in approximately the same month of each year to control for seasonal effects [22]. Endoscopies were postponed or cancelled if the participants had experienced diarrhoea or had taken antibiotics or non-steroidal anti-inflammatory drugs (NSAIDs) within one month before the scheduled endoscopy, or were pregnant or lactating. As the trial lasted 3 years and 4 months, participants underwent endoscopy on between one and four occasions.

In the second set, participants who experienced an episode of diarrhoea lasting for 3 days or more were invited to undergo endoscopy during that episode and when free of diarrhoea (referred to as 'diarrhoea biopsies'). Sometimes, by chance, a scheduled biopsy had been taken within a month of the onset of the diarrhoeal illness, but more commonly a convalescent biopsy had to be taken a month after resolution of the illness (both of these together are referred to as 'convalescent biopsies'). The subsequent course of this illness was noted and illnesses were divided into acute (less than 14 days), persistent (14 days or more), and dysenteric (bloody diarrhoea, irrespective of duration).

In all cases, age, sex, HIV status and CD4 count (if HIV seropositive) were recorded for each participant at the time of each biopsy. HIV testing was carried out using Capillus rapid kits and CD4 count using FACScount (Beckton Dickinson). We measured nutritional status by measuring height, weight, and mid upper arm circumference (MUAC), and body mass index (BMI) calculated in the usual way.

Analysis of intestinal biopsies

Four biopsies were collected using standard biopsy forceps and placed in sterile Cryovials (Nalgene, Rochester, NY) before immersion in liquid nitrogen and storage at $-80°C$. Within 6 months two biopsies were retrieved and digested together in TRI Reagent (Sigma-Aldrich, Gillingham, Dorset, UK), followed by phenol-chloroform-isopropanol extraction, using standard procedures, and PCR for the α-defensins. Where the initial amplification was unsatisfactory, and for amplification of β-defensins, LL-37, and housekeeping genes, the remaining biopsies were used. Total RNA was reverse transcribed to cDNA using reverse transcriptase (Promega, Southampton, UK). mRNA for human α defensins 5 and 6 (HD5, HD6), human β-defensins 1 and 2 (hBD1, hBD2), LL-37 and interleukin (IL)-8 were quantified by real time PCR using the primers and conditions listed at http://www.qmul.ac.uk/Profiles/Digestive%20Diseases/Kelly%20Paul.htm, with SyBR-Green as fluorophore and using a Corbett RotorGene 3000 thermal cycler (Corbett Research, Sydney, Australia). In order to be able to quantify HD5 and HD6 mRNA in absolute terms, each run included a series of standards which comprised plasmids into which the target sequence of interest had been cloned [17], and a standard cDNA was included in all runs and used as an additional reference gene. The standard cDNA was made from RNA extracted from Caco-2 cells treated with 20ng/ml IL-1β (Peprotech, London, UK). For hBD1, hBD2 and LL-37, the invariant epithelial marker cytokeratin-19 (CK-19) was used as a reference marker of epithelial cell mass and relative quantification performed; for LL-37 GAPDH was also used. In preliminary experiments, $α_1$-anti-trypsin was used as an invariant Paneth cell marker [17] to confirm that changes in HD5 and HD6 expression were not due to changes in Paneth cell mass (data not shown). Where any unsatisfactory results were obtained (impure RNA

preparation, low yield, poor amplification, poor melt curves), the whole protocol was repeated beginning with the reserve biopsies.

Paneth cell granule abnormalities

We have previously described Paneth cell granule depletion in Zambian adults which appeared to be associated with low serum zinc concentrations [23]. During endoscopy, two additional biopsies were collected (making 6 in total) and orientated before fixation as previously described [24]. We evaluated Paneth cell morphology in 217 satisfactorily-orientated haematoxylin-eosin stained sections using the same criteria as in the previous study (i.e. normal, mild granule depletion, severe granule depletion)[23].

Measurement of serum leptin concentration

When it became apparent that there were sex-dependent nutritional interactions with antimicrobial gene expression, serum leptin and adiponectin concentrations were measured in sera obtained from blood collected at the time of endoscopy. Leptin and adiponectin were assayed by ELISA (R&D Systems, Abingdon, UK) according to the manufacturer's instructions.

Data analysis

HD5 and HD5 mRNA content was expressed as log number of transcripts per microgram of total RNA extracted, using standard curves constructed using the plasmid standards. For hBD1, hBD2 and LL-37, all results were expressed relative to CK-19 and the Standard cDNA by first subtracting CtSample from CtStandard to derive the variable x and then subtracting CtRef from CtStandard to derive the variable y, where CtRef is the Ct value of amplification of the reference gene (CK19), CtStandard is the Ct of the Standard cDNA, and CtSample is the Ct value of amplification of the gene of interest. The ratio of expression of the gene of interest to CK-19 was obtained using the formula $2^{(y-x)}$.

The primary comparison was mRNA of these five genes in scheduled biopsies from recipients of MM or placebo, and this was analysed using the Kruskal-Wallis test. hBD2 expression was detected in a minority of participants so it was analysed as a categorical value (either present or absent). In exploratory analysis, sub-groups were analysed by age, sex, HIV status, CD4 count, and nutritional status. For the diarrhoea samples, the primary analysis was the difference in mRNA between diarrhoea and convalescent biopsies, and this difference was compared in MM and placebo recipients using the Kruskal-Wallis test. Comparison of HD5 across quartiles of BMI was analysed using Cuzick's non-parametric trend test. Correlations between nutritional parameters and defensins expression used Spearman's rank correlation coefficient. Statistical analysis was carried out using Stata 10 (Stata Corp, College Station, TX).

Sample size calculations were based on results of a previous study of α-defensins [22] which revealed significant effects in predicting diarrhoea of just under one log transcripts/μg total RNA for HD5. We estimated that to detect an effect of the size of 0.5 log transcripts/μg total RNA (from 3.3 to 2.8) with SD of 0.98 with 90% power and 95% confidence would require 81 in each group for each antimicrobial gene product. The sample size for α-defensins was much higher than this to ensure that the data were representative and to allow for sub-group analysis.

Results

The trial was carried out from September 2003 to December 2006. Demographic and other characteristics of participants from whom biopsies were obtained are given in Table 1, and their flow through the trial is shown in Figure 1.

Impact of MM supplementation on gene expression in scheduled samples

A total of 511 scheduled sets of biopsies were taken from 287 participants. After the cross-over, 130 of these participants were still in the trial and 165 of these biopsies were taken after the cross-over. mRNA was assayed by real time RT-PCR for HD5 (n=476), HD6 (n=479), hBD1 (n=136), hBD2 (n=197), and the cathelicidin LL-37 (n=196). Fewer results were obtained for β-defensins and LL-37 as cDNA was used up by the PCRs performed. The α-defensins and hBD1 were constitutively expressed but hBD2 was expressed in only 10% of biopsies and LL-37 in 40%. Interleukin (IL)-8 mRNA was measured to determine if intestinal inflammation explained the expression of hBD2, which is an inducible defensin, but no difference was found between biopsies in which hBD2 was detected and those in which it was not (P=0.41). In contrast, IL-8 mRNA (relative to the cytokeratin CK-19) was higher (median 7989, IQR 7224-8303) in 70 biopsies in which LL-37 was found than in 110 biopsies in which LL-37 was not detected (7738, 7222-7960; P=0.001). Overall, there was no statistically significant difference between HD5, HD6, hBD1, hBD2, or LL-37 in the MM group compared to placebo (Table 2). When comparing the change in α-defensin expression at the cross-over, there was no difference by treatment allocation (P=0.29 for HD5, P=0.35 for HD6). Neither was there any difference by HIV status (data not shown). In this study there was no correlation between the expression of HD5 and probability of diarrhoea in the 2 month period of follow up after the biopsy was taken.

Sub-group analysis of antimicrobial gene expression

There was no difference in gene expression by HIV status, CD4 count or age, but there was evidence of an effect of sex and body mass. HD5 expression (median, IQR log transcript number per μg total RNA) was lower (P=0.04) in 280 biopsies from women (3.2, 2.5-3.9) than in 196 from men (3.5, 2.7-4.1). hBD1 mRNA (relative to CK-19 mRNA) was higher (9747, 9260-10057) in 19 biopsies from participants with BMI<18.5kg/m^2 than in 110 biopsies from participants with higher BMI (8939, 0-9846; P=0.03).

Analysis of sub-groups as defined above revealed two sub-groups in which there was an effect of treatment allocation. The first was an effect on HD5 in participants whose BMI was 18.5kg/m^2 or less (Figure 2). The second was an effect on hBD1 mRNA in HIV seronegative participants: median (IQR) was 9706 (0 – 10229) in 41 participants allocated to MM compared to 6339 (0-9515) in 36 participants allocated to placebo (P=0.01).

HD5 fell with quartiles of increasing BMI in women and in participants receiving MM, with the strongest effect in women allocated to MM (P=0.001; Figure 3). Spearman's rank correlation coefficients between HD5 or HD6 and BMI or MUAC showed consistent relationships (Table 3). No such relationships were evident for hBD1, hBD2 or LL-37 (data not shown).

Adipocytokines have effects on inflammatory responses [25,26] and display sex-dependent correlations with nutritional status in this [27] and other populations. We looked for evidence that they might signal body mass to Paneth cells by measuring concentrations in blood by ELISA. Serum leptin (median, IQR) was 11.3 (.6-27.4) ng/ml in 162 women and 2.5 (1.3-4.0) ng/ml in 79 men (P=0.0001). Serum adiponectin was 14.2 (10.2-21.1) mg/ml in 83 women compared to 10.8 (5.6-24.3) in 48 men (P=0.19). Serum leptin showed strong correlations with BMI and MUAC, especially in women (ρ=0.71 and 0.69 respectively;

P<0.0001 for both) as expected, but the relationship between adiponectin and BMI was only just significant (ρ=-0.26; *P*<0.02) and there was no correlation with MUAC. While HD5 and BMI were correlated in women taking micronutrients, the correlation was weaker for leptin and there was no correlation for adiponectin (Table 3). Furthermore, in multiple linear regression, leptin was not significantly correlated with HD5 if BMI was also included in the model. We conclude that neither leptin nor adiponectin explain the correlations between nutritional status and α-defensin gene expression.

Paneth cell granule depletion

Evidence of Paneth cell granule depletion was looked for in 217 biopsies. Mild depletion was seen in 30 participants allocated to receive MM supplements and 36 allocated to placebo; severe depletion was seen in 22 and 12 respectively. None of these differences were statistically significant even after Mantel-Haenszel stratification for low BMI.

Impact of MM supplementation on gene expression changes during diarrhoeal disease

We also studied HD5 and HD6 expression in the dynamic circumstances before, during or after diarrhoeal disease in 54 participants: 34 with acute diarrhoea, 15 with persistent diarrhoea and 5 with asymptomatic infections (3 *Cryptosporidium parvum*, 1 *Salmonella enterica* serovar typhimurium, 1 *Strongyloides stercoralis*). Suppression of HD5 or HD6 by intestinal infection was inferred when the value during diarrhoea was >0.5 log less than the pre-post diarrhoea sample. Suppression of HD5 was observed during 44% of episodes and suppression of HD6 in 46% of episodes. During diarrhoea, HD5 expression was reduced by 0.8 log transcripts/μg total RNA in placebo recipients (*P*=0.02) but not in MM recipients or after the cross-over (Figure 4).

Discussion

In tropical populations, where micronutrient deficiencies are widespread and infectious diseases are common, understanding interactions between nutrition and host defence could be of great importance. We set out to test the hypothesis that micronutrient supplementation can bolster innate defence of the intestinal mucosa. Our trial demonstrates that micronutrient supplementation does not augment expression of antimicrobial genes in the gut, at least in the dose range examined. Thus, the population difference we have previously reported between European and African populations [14] is unlikely to be explained by borderline micronutrient deficiencies, unless adults in this population for some reason require higher intakes to achieve 'healthy' micronutrient balance (which might in fact be the case [18]). However, in malnourished adults HD5 was up-regulated by the supplementation. Although this is a subgroup analysis, the study cohort included 48 malnourished adults, a not insubstantial number. The opportunity for subgroup analysis arises from the large sample size in this study, and it is precisely the subgroup in which one would have expected, *a priori*, the benefit of micronutrients to matter. Most importantly, our data also suggest that defensin expression is reduced during acute infectious diarrhoea, and that MM supplementation can reduce the impact of pathogens.

HD5 and HD6 mRNA levels were correlated with nutritional status, which is of interest because HD5 is probably the most abundant of the small intestinal antimicrobial peptides [17]. No such correlations were observed for the other genes analyzed but the power of the correlation analysis for other mRNAs is limited as fewer samples were available for quantification. The observed sex difference was unexpected. Our findings suggest that there are sex-dependent, possibly endocrine, determinants of HD5 and HD6 expression. We have previously found that in AIDS patients in Zambia, serum leptin does not correlate with fat mass in men but does in women [27]. In the light of the findings reported here, we re-

analysed data from two previous community studies [18,28] and found that the incidence of diarrhoea was higher in women. We had previously reported [22] that lower HD5 expression predicts diarrhoea, although in this study we could not confirm this when analysed in the same way as in the earlier report [22]. The lower HD5 expression in women, who also had higher diarrhoea incidence, may provide some evidence that HD5 contributes to host defence against diarrhoea-causing pathogens. Recent data suggest that the impact of zinc on mortality in children in Tanzania is sex-dependent [29,30] and evidence from Guinea-Bissau indicates that the response to vitamin A supplementation may also be sex-dependent [31]. Further work is needed to determine the mechanism of the sex-dependent relationship between host defence and nutritional status. We did not find evidence that leptin or adiponectin correlate as closely with HD5 gene expression as does BMI, so we conclude that these molecules are unlikely to be the molecular signals of body composition to the Paneth cell. It has recently become apparent that leptin and adiponectin are also synthesised by Paneth cells [32] but their biological function when secreted by the Paneth cell is as yet unexplained.

The level of HD5 and HD6 gene expression was expressed in absolute terms as mRNA transcripts per μg total RNA, and was considerably lower than in published studies from our and other groups [14,17]. We cannot explain this difference which arose when we changed from competitive RT-PCR [14] to real time RT-PCR. We have re-calibrated the plasmid standards, which were used throughout, and a cDNA control was used to ensure consistency between runs over the 3.3 years of the study, so we are confident that our data are internally consistent and the differences and correlations we found are meaningful.

LL-37 is constitutively expressed in the large but not the small intestine. Here, its expression was detected in a minority of small intestinal biopsies. As the MM supplement includes vitamin D we looked for any evidence of induction of LL-37 in the MM group, but this was not seen. Vitamin D up-regulates LL-37 transcription in biliary epithelial cells in culture [33], but this effect has not been investigated in small intestinal epithelial cells. In colonocytes, butyrate induces expression of both the vitamin D receptor [34] and LL-37 [35], so on a high-carbohydrate African diet (with ample butyrate generation in the colon) we might have expected LL-37 expression to be responsive to vitamin D, but it is unknown if these transcriptional effects operate over long periods of time. LL-37 expression was associated with higher IL-8 expression, suggesting that its expression is partially induced by inflammatory signals. As LL-37 is also expressed by neutrophils, it is possible that this is due to neutrophil recruitment by IL-8. This aspect of LL-37 regulation needs further investigation *in vitro* and *in vivo*. Work by other groups [36] led us to expect that hBD2 and IL-8 expression would be associated, but hBD2 was expressed in only 10% of the biopsies we analysed and was unrelated to IL-8 expression.

HIV seroprevalence is around 22-30% in urban Zambia [37], and a high proportion of our participants were infected with it. However it does not seem to have an impact on antimicrobial peptide expression in the intestine. This lack of effect of HIV is entirely consistent with our previous findings [22], and it offers the hope that it might be possible to improve host defence in the gut through antimicrobial peptides even when cellular immunity is severely impaired.

Acknowledgments

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                                              Resident in study area
                                                     n=733

                      Declined
                       n=233

                                                  Randomised
                                                    n=500

        Underwent permeability testing not endoscopy
                       n=213

                                               Underwent endoscopy
                                                     n=287

                     First phase placebo                        First phase MM
                           n=145                                    n=142

       Withdrew or died                       Withdrew or died
            n=76                                   n=70

                     Second phase MM                           Second phase placebo
                          n=69                                      n=62
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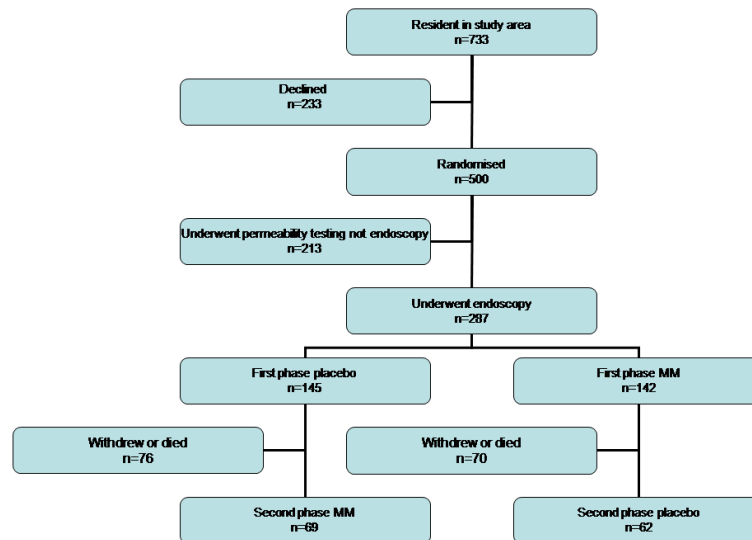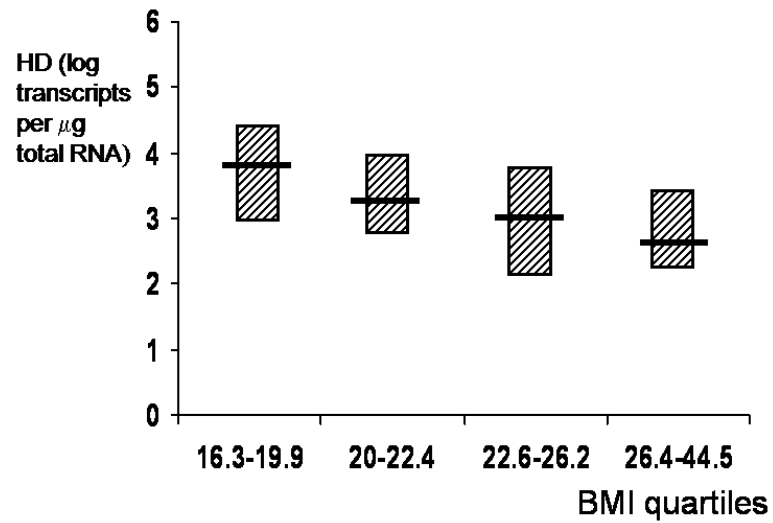
Figure 1.
Flow of participants through the trial. Of the 500 participants randomised, 287 underwent endoscopy at one time or another. Others were designated to participate in a parallel sub-study of permeability which will be reported elsewhere, or were ineligible for either investigation (for example, pregnancy or having taken non-steroidal anti-inflammatory drugs).
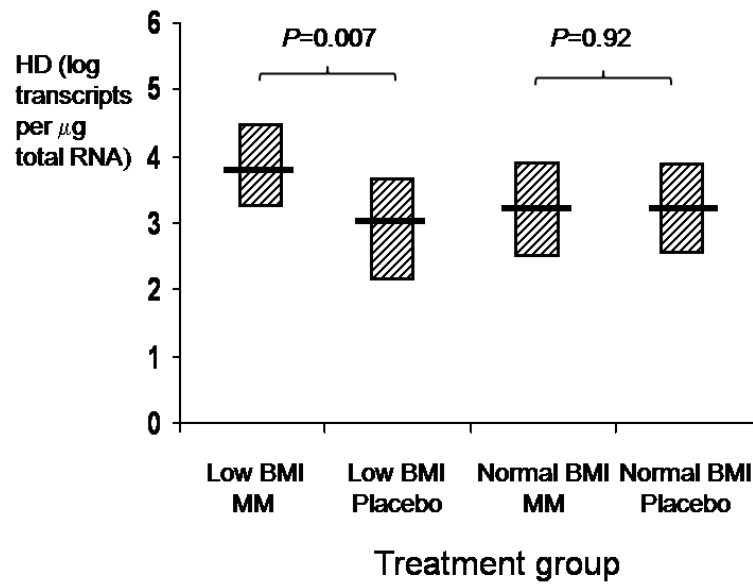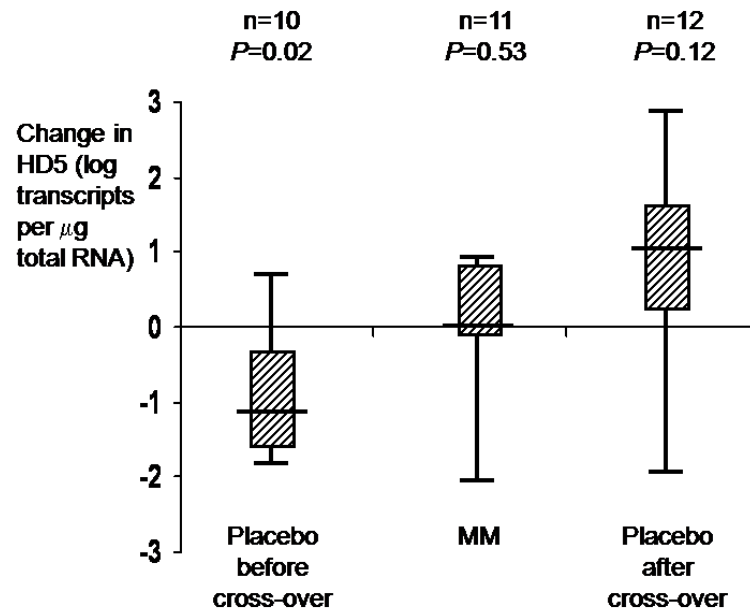
Figure 2.
Effect of multiple micronutrient supplementation in malnourished (BMI<18.5kg/m^2) adults allocated to MM (n=18) or placebo (n=30), and in adults of normal (BMI>18.5kg/m^2) body mass index allocated to MM (n=219) or placebo (n=209). Bars show median values and shaded boxes the interquartile range (IQR). P values refer to Kruskal-Wallis test.

Figure 3.
HD5 expression (median, IQR) by quartiles of BMI in women allocated to micronutrient supplementation only. As BMI increased, HD5 mRNA decreased ($P<0.001$ using Cusick's non-parametric test for trend), prompting an analysis of adipocytokine concentrations in serum.

Figure 4.
Changes in HD5 expression (median change, IQR, range) during diarrhoea, shown relative to the convalescent biopsy (see Methods) expressed so that a negative value means down-regulation during the episode of diarrhoea. In patients allocated to placebo there was a significant down-regulation during diarrhoea, but this was not seen in patients allocated to micronutrients (MM), nor to those allocated to placebo after the cross-over. This could be due to carry-over of a protective effect despite the 3 months washout.

Table 1

Demographic and other characteristics of participants from whom biopsies were taken

	Before cross-over			After cross-over		
	MM	Placebo	P	MM	Placebo	P
Number of participants	127	132		68	62	
Number of biopsies	171	175		85	80	
Age (mean, SD) in yrs	36.4 (12.2)	34.8 (14.4)	0.04	36.8 (13.0)	38.4 (11.8)	0.26
Sex Male	47	53	0.60	25	31	0.13
Female	80	79		43	31	
BMI (kg/m^2) (median, IQR)	21.3 (19.6-23.6)	21.5 (19.6-25.2)	0.38	22.6 (20.2-26.6)	21.5 (19.1-24.0)	0.05
Proportion with BMI <18.5kg/m^2	8/127 (6%)	15/132 (11%)	0.15	6/66 (9%)	9/61 (15%)	0.41
MUAC (cm)	26.2 (24.2-27.5)	26.2 (24.3-28.5)	0.42	29.0 (25.8-31.0)	26.5 (24.5-29.5)	0.01
HIV positive	51/116 (44%)	41/121 (34%)	0.14	26/65 (40%)	24/60 (40%)	1.0
CD4 count (cells/µl) (median, IQR)	315 (200-426) in 50 participants	350 (233-474) in 40 participants	0.31	374 (209-504) in 24 participants	345 (259-479) in 24 participants	0.91

Table 2

Antimicrobial gene expression in scheduled biopsies and serum leptin by treatment allocation (MM or placebo)

Gene	n	Whole group	MM	Placebo	P
HD5 (median, IQR) log transcripts/μg total RNA	476	3.3 (2.6-3.9)	3.4 (2.6-3.9)	3.2 (2.6-3.9)	0.48
HD6 (median, IQR) log transcripts/mg total RNA	479	3.8 (2.9-4.4)	3.8 (2.9-4.5)	3.8 (2.9-4.4)	0.45
hBD1 (relative to CK19)	136	9258 (0-9893)	9417 (0-9890)	8613 (0-9894)	0.22
hBD2 (n (%) detected)	197	20 (10%)	9/99 (9%)	11/98 (11%)	0.65
LL37 (n (%) detected)	196	78 (40%)	39/97 (40%)	39/99 (40%)	1.00
IL-8 (relative to CK19)	188	7792 (7223-8154)	7799 (7236-8157)	7792 (7221-8150)	0.63
Leptin (ng/ml)	241	5.6 (2.5-17.4)	5.5 (2.2-17.4)	5.7 (2.8-18.0)	0.88
Adiponectin (mg/ml)	131	13.8 (9.0-21.8)	13.2 (8.7-23.8)	14.0 (9.2-23.8)	0.46

Table 3

Correlations between a-defensin expression and nutritional measures

Spearman's rank correlation coefficient	HD5	*P*	HD6	*P*
Correlation with BMI				
Women only (n=249)	−0.21	0.0007	−0.20	0.001
MM only (n=202)	−0.26	0.0002	−0.17	0.01
Women taking MM (n=116)	−0.32	0.0002	−0.30	0.0005
Correlation with MUAC				
Women only (n=248)	−0.24	0.0001	−0.16	0.02
MM only (n=203)	−0.29	0.0001	−0.12	0.14
Women taking MM (n=134)	−0.35	<0.0001	−0.23	0.007
Correlation with serum leptin				
Women only (n=154)	−0.17	0.04	−0.20	0.01
MM only (n=109)	−0.12	0.19	−0.07	0.47
Women taking MM (n=80)	−0.20	0.07	−0.22	0.05
Correlation with serum adiponectin				
Women only (n=80)	0.13	0.24	0.003	0.98
MM only (n=62)	0.13	0.32	0.13	0.32
Women taking MM (n=44)	0.14	0.38	0.06	0.70