Proceedings of the Nutrition Society (2010), **69**, 261–268 doi:10.1017/S0029665110000108
 First published online 5 March 2010

The Summer Meeting of the Nutrition Society was held at the University of Surrey, Guildford on 30 June–2 July 2009

Conference on 'Over- and undernutrition: challenges and approaches'

Symposium 4: Gut function: effects on over- and undernutrition Nutrition, intestinal defence and the microbiome

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The interaction between nutrition and infection was the subject of important work by several groups in the 1960s. The explosion of knowledge in immunology, including innate immunity, has led to increased understanding of the impact of nutrition on host defence, but much more work needs to be done in this area. In the last decade an increasing volume of work has opened up the previously obscure world of human endogenous flora. This work suggests that the microbiome, the total genetic pool of the microbiota, contributes to the already complex interaction between nutrition and infectious disease. The established concept that nutritional status, host defence and infection all impact on each other now has to be expanded into a multiple interaction, with the microbiota interacting with all three other elements. There is good evidence that the microbiome programmes host defence and drives a metabolome that impacts on energy balance, and indeed on some micronutrients. In turn, host defence shapes the microbiome, and nutritional status, particularly micronutrient status, helps determine several elements of host defence. While interventions in this area are in their infancy, the understanding of interactions that already have an enormous impact on global health is now at a threshold. The present review explores the evidence for these interactions with a view to putting potential interventions into the context of a conceptual framework.

Nutrition: Intestinal defence: Microbiome

In the 1960s seminal work (for example, see Scrimshaw *et al.*[1] and Mata[2]) defined the concept of the nutrition–infection interaction[3]. Understanding of the details of this interaction is still sketchy, but now there are new microbiological insights to fit into the picture. Although Metchnikof, who discovered the macrophage, formed the belief over one century ago that man needs a proper bacterial environment, and that 'good' bacteria may actually promote health[4], he was in no position to understand the remarkable dynamics of the balance between human hosts and their microbial world, and the microbiome is what drives its activities. It is only now, in the early twenty-first century, that researchers are really in a position to understand the ecosystem that man inhabits and that inhabits man. Only now is it possible to begin to understand that nutrition influences the microbiome and that the microbiome influences nutrition, that both these forces influence host defence and that host defence may be one mechanism through which the other forces influence each other. The present review explores emerging evidence that nutrition, host defence and the microbiome interact (Fig. 1) in a way that possibly in future could be exploited to the benefit of human health.

What is the microbiome?

Man lives in a microbial world and it is increasingly recognised that the human organism is host to a very large microbial community, which colonises every surface of the body: skin; mouth; gut[5]; vagina. Even within each of these surfaces there is diversity; the skin of the axilla is host to a very different microbial community from that of the skin of the forearm or perineum and the stomach has a different microbial flora from that of the mouth or rectum. Man is therefore in a sense the sum of many parts; in a much-quoted estimate an individual's own cells are outnumbered by the microbial residents of that individual

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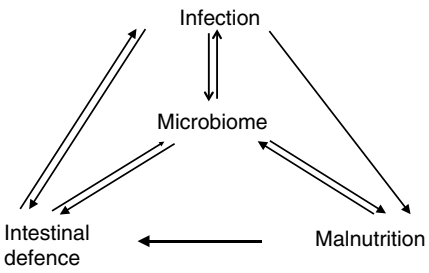

Fig. 1. The interaction between nutrition, intestinal defence, infection and the microbiome. The microbiome is the gene pool of the gut microbiota, which in its role as metabolic organ and as modulator of immune responses plays a key role in the interactions between the other elements. Note that two of these interactions are one way, so that any influence of intestinal defence on nutritional status must be indirect; likewise, the impact of nutritional status on susceptibility to infection operates through host defence or through effects on the microbiome.

by possibly an order of magnitude and their genetic material is dwarfed by that of the microbiota. The microbiome is an analogy with the genome, which is the sum of man's germ line-derived genetic material. Understanding the microbiome is just beginning now that new techniques are available for understanding it without the need for bacterial culture of each species of the microbiota[6–8]. The reliance until very recently on culture to identify bacteria has always been a problem, as many of the bacteria present cannot be cultured. Culture has a further problem; it yields information on the biochemical and growth characteristics of the species present, but that is not necessarily the required information. Certainly, from a nutritional point of view what is of more interest is the sum of the metabolic activities of the flora (the 'metabolome') or the composition of the total gene pool, which represents man's genes and that of the microbiota (the 'metagenome'). Man has recently been released from a dependence on bacterial culture by the development of advanced DNA sequencing and hybridisation methods that are outside the scope of the present article, but which allow quantification of the relative abundance of different bacteria at a genetic level in a way that is much more representative of the total bacterial pool than has ever been possible before.

Bacterial taxonomy is complex and rapidly evolving, but generally it is possible to divide the bacterial kingdom into about seventy major divisions (each of which corresponds to a phylum), twelve of which are represented in the human gut[6]. In the intestine ten of these phyla have been described (Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, Fusobacteria, Verrucomicrobia, Cyanobacteria, TM7, Spirochaetes, and VadinBE97). In the stomach eight phyla are present, although in much lower abundance (Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria, Fusobacteria, TM7, Deferribacteres, and Deinococcus-Thermus). The flora of the oral cavity is different again. Probably 15 000 species are represented in the human gut, so description of the microbiome at a species level is an impossibly complex task and currently changes in the abundance of major phyla are all that can reasonably be encompassed. In addition to bacteria there are viruses (including phages that parasitise bacteria), yeasts and other fungi, protozoa and helminths. All these microbes, even helminths when in low abundance, can be considered to be commensals. There is also one dominant representative of the primitive prokaryotes, the Archaea, in *Methanobrevibacter smithii*.

In the gut this vast microbial community is not inactive. It carries out several important functions including fermentation and catalysis of complex polysaccharides (particularly those containing xylan, pectin and arabinose residues), deconjugation of bile salts, salvaging urea and synthesising essential amino acids[9] and vitamin K; there are probably many others. Together, the collection of metabolic activities in a non-host compartment makes the flora of the gut a separate metabolic organ or 'metabolome'.

One such effect is the trophic effect that the flora has on intestinal tissue in the host. The SCFA liberated by polysaccharide breakdown in the right colon of human subjects have effects on colonic integrity as they represent an important metabolic fuel for the colonic epithelium[10]. There is also increasing evidence from animal models that the microbiota is required for proper development of the gastrointestinal tract and associated immune cells (see later).

The establishment of the microbiota varies greatly depending on circumstances, but once established it appears to be remarkably stable. *In utero* the gut is sterile but after birth it is rapidly (in <3 d) colonised by microbial populations. Infants born by caesarean section have a different flora to those born vaginally, with delays in colonisation following caesarean section of 10 and 30 d for Lactobacillus-like and Bifidobacterium-like species respectively[11]. The implications for human health are as yet unknown. These findings have been confirmed and extended by an analysis from a Dutch birth cohort, in which mode of delivery, breast- or formula feeding and the use of antibiotics were all found to be major determinants of the composition of the flora[12]. Antibiotics can have long-lasting effects on the microbiota; deep sequencing of the microbiome from three adults before and after ciprofloxacin treatment has shown that most taxa recover by 4 weeks, but some taxa take much longer to recover[13]. Similar results have been obtained in mice[14], in which it has also been shown that an intestinal pathogen (in this case *Citrobacter rodentium*) perturbs the microbiota but the pretreatment status quo returns within 28 d[15]. Importantly, the microbiome tends to recover a composition characteristic of that individual even after treatment with a broad spectrum antibiotic, despite the apparent opportunity that this treatment represents for a change in the flora and that is sometimes exploited by *Clostridium difficile*[16].

Most interesting of all, there are several examples of the ability of the microbiota to transmit a phenotype from one experimental host to another. Mice that are deficient in the transcription factor T-bet develop colitis. T-bet defines T-cells of the T-helper 1 subset, which are pro-inflammatory T-helper cells that are important in the clearance of viral and other intracellular infections and typically secrete the cytokine interferon-γ. By transferring the colonic flora from mice with T-bet deficiency and colitis to wild-type animals the colitis can be transferred too, suggesting that the flora has become 'colitogenic'[17].

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https://doi.org/10.1017/S0029665110000108 Published online by Cambridge University Press

In a second example, mice deficient in the leptin gene develop obesity and transferring their flora to wild-type mice also transfers a change in energy balance so that the recipient mice gain increased fat[18]. This outcome can be referred to as a 'transplantable metabolome' and it may have enormous implications for understanding human obesity, as one of the changes that can be seen in the microbiome of the *ob/ob* (leptin deficient) mouse is an increase in Firmicutes : Bacteroidetes, which has also been described in obese human subjects[19]. It is possible that an obesity-generating diet in some way transforms the metabolic activity of the microbiome to make obesity more inevitable, and it can be speculated that this effect might help explain why cycles of dieting often fail to control obesity and why some individuals do appear to be condemned to sustain higher body mass than others, even when consuming less energy.

The microbiome influences host defence

As mentioned earlier, the intestinal flora appears to be required for development of the gut and for full maturation of the mucosal immune system. Experiments in germ-free animals (which are raised in a sterile environment and have no endogenous flora) are instructive[20]. Such animals have reduced mucosal thickness and underdeveloped mucosal immune systems with hypocellular lymphoid tissue (Peyer's patches, isolated lymphoid follicles and intraepithelial lymphocyte and lamina propria plasma cell compartments are all less cellular). Mesenteric lymph nodes are hypoplastic and there is a specific reduction in $CD4^+CD25^+Foxp3^+$ regulatory T-cells. Production of IgA (the dominant secreted Ig class in the gut) is reduced, probably because secretion of IgA seems to require the phagocytosis and carriage of bacteria of the flora from the epithelium to the mesenteric lymph node[21]. Expression of certain molecules fundamental to innate immunity (Toll-like receptor 9, angiogenin 4 and RegIIIγ) and adaptive immunity (MHC class II, IL-25) is reduced[20].

Studies using experimental animals with specific gene deletions confirm the dependence of the development of the gut on the microbial flora. Animals with deletions of the gene that encodes MyD88 (a central signalling molecule of innate immunity), and that cannot therefore recognise the presence of the microbial flora, develop spontaneous colonic inflammation[22]. When this deletion is specific to Paneth cells, which are the cellular source of α-defensins, the endogenous secretion of antimicrobial peptides is impaired, indicating the importance of the gut flora for maintaining constitutive expression of antimicrobial peptides and host defence against pathogens[23]. One molecule that can confer protection from inflammation has recently been shown to be the polysaccharide A from *Bacteroides fragilis*[24]. In another example expression of the antimicrobial peptide RegIIIγ is reduced in germ-free mice but can be restored by conventional flora[25]. Colonisation with the single species *Bacteroides thetaiotaomicron* does not fully restore expression in wild-type animals, but in IgA-deficient animals this single species fully restores RegIIIγ expression, suggesting that in the absence of IgA the close association between bacteria and epithelium drives constitutive expression of the antimicrobial molecule[25].

The microbiome influences nutrition

The transplantable metabolome observations referred to earlier[18] strongly suggest that the microbiome can influence nutrition. Further evidence from an animal model may have identified one mechanism by which the flora influences fatty acid metabolism. SCFA are ligands for a G protein-coupled receptor, Gpr41, present on enteroendocrine cells in the colon that release the gut hormone peptide YY, which in turn drives SCFA absorption[26]. Gpr41-knock-out mice are leaner than wild-type animals, suggesting that energy salvage from SCFA is less efficient without this receptor, but this difference in adiposity is only observed in gnotobiotic (i.e. known composition, in this case *Bacteroides thetaiotaomicron* and *Methanobrevibacter smithii*) animals and not in germ-free animals[26]. This key paper demonstrates that the flora interacts with host physiology to modulate host nutrition, and the mechanism is beginning to be elucidated. Undoubtedly, over the coming years further data will elucidate the details of this fascinating interaction.

Nutrition influences host defence

It has become axiomatic in teaching physicians, nurses and dietitians that malnutrition leads to increased susceptibility to infectious disease. The term 'malnutrition' in this context is generally considered to mean reduced body mass. However, the evidence base for this apparently simple statement is slender. In classic experiments on starvation in human volunteers[27] no evidence was found that incidence of infectious disease is higher in the volunteers than in the carers who looked after them, despite severe depletion of body cell mass. Furthermore, there is no evidence that patients with anorexia nervosa have an increased incidence of infections[28], although case reports exist of exotic, particularly fungal, infections that appear to complicate this disorder. In one of these studies IL-2 synthesis was reduced by 49%[29]. In another study T-lymphocyte populations were found to be normal, with lymphocyte proliferation in response to phytohaemagglutinin and concanavalin A showing a marginal increase[28]. Conversely, another study has observed high circulating levels of IL-1β and TNFα, together with reduced T-cell activation as expressed by CD2 and CD69[30]. While this report is one of very few to identify an immunological change, it may not be important if an increase in susceptibility to infection is not observed. These examples are of 'pure' or primary undernutrition, a result of simple energy deprivation rather than undernutrition secondary to disease. Thus, altogether there is very little evidence that pure malnutrition influences host defence mechanisms.

In a more complex situation, in a trial in which Kenyan schoolchildren were randomised to several different food supplementation regimens (meat-based, milk-based, vegetable oil-based or none) antibody titres to *Helicobacter pylori*, rotavirus, tetanus toxoid and malaria merozoite

https://doi.org/10.1017/S0029665110000108 Published online by Cambridge University Press

surface proteins were found to show very little change[31]. In severe malnutrition in children there is good evidence of atrophy of the thymus and lymphatic tissues[32–34], and an interesting hypothesis has been put forward that thymic impairment programmed in early life carries forward into susceptibility to infection in adult life[35]. More recently, dendritic cell function has been shown to be markedly impaired in severely-malnourished children[36] but these studies nicely illustrate the difficulty of interpreting data in severe clinical malnutrition. The most recent study has shown that lipopolysaccharide is correlated most closely with the immune impairment[36], suggesting that it is the coexistent infections that do the real immunological damage. In 'pure' primary malnutrition, because of pure energy deprivation as in famine and conflict situations, survival is better at extreme low BMI than would be expected in patients with malnutrition complicated, or induced by, infectious or malignant disease[37].

However, as there is abundant evidence that malnourished children in developing countries have a high coexistent burden of infectious disease[38], how is this situation explained? There appear to be two possible explanations. One, as suggested earlier, is that much of the immunodeficiency in severe malnutrition is a consequence of, as well as a cause of, infection. The second is that the immunodeficiency seen in severely-malnourished patients is related to micronutrient deficiencies. There is much better evidence for the hypothesis that micronutrient deficiencies impact on host defence than for the hypothesis that reduced body cell mass (energy deprivation) impacts on host defence. This second hypothesis would fit with the lack of evidence for substantially increased infection susceptibility in pure malnutrition, as micronutrient depletion in anorexia nervosa, for example, would be in a sense in proportion to the reduced body cell mass until an inflammatory response supervenes, when losses of micronutrients as a result of inflammation[39] would precipitate immunological consequences. The evidence that micronutrients can alter host defence in the gut will be reviewed, with an emphasis on human studies and a focus on four dominant elements of host defence in the intestine: physico-chemical barriers; innate immunity; humoral immunity; cell-mediated immunity. The micronutrients for which there is the most compelling evidence are vitamin A and Zn, and also briefly discussed are Se, other antioxidants and Fe. In much of what follows selected evidence has been used that does not directly relate to intestinal defence mechanisms because direct evidence for an effect in the gut is scant.

Vitamin A

There is strong evidence from randomised controlled trials in Ghana and Indonesia that vitamin A has important effects in reducing adverse outcome from infectious disease in underdeveloped countries, particularly diarrhoea and measles[40]. However, it is not clear whether the effect is on immune function or on some other aspect of host defence such as epithelial integrity, and the evidence for an immune booster effect in human subjects appears to be mixed. To summarise a recent excellent review[41], there is good evidence that intestinal epithelial integrity is improved by vitamin A, but not of improved antimicrobial properties in breast milk, and there is no evidence of improved barrier function in the vagina. There is very preliminary evidence of reduced secretion of TNFα and IL-6 when challenged by specific pathogens. There is some evidence of a beneficial effect in raising CD4 counts in children infected with HIV but not in adults. Furthermore, there is no conclusive evidence of effects on cytokine production or lymphocyte function, but antibody responses to tetanus toxoid may be enhanced if the vitamin A is given before the vaccine. When contrasted with the highly significant effects of vitamin A in reducing childhood morbidity and mortality, particularly from measles and diarrhoea, the very uncertain evidence of effects on immune competence is striking. It seems likely on the basis of current evidence that epithelial or barrier integrity is an important part of the effect of vitamin A. However, two recent studies on HIV infection in adults[42,43] have cast doubt on the safety of vitamin A supplementation, which was reported to be associated with increased mortality.

Zinc

There is abundant evidence that Zn is a critically-important nutrient for the intestinal defence. Zn is effective in the treatment of diarrhoea; in a recent meta-analysis a reduction in the duration and severity of acute and persistent diarrhoea is confirmed[44]. Zn also gives a 42 (95% CI 10, 63) % reduction in treatment failure or death from diarrhoea[45]. Zn supplementation is now recommended by the WHO alongside oral rehydration salts during treatment of acute diarrhoea in children. However, Zn does not reduce all-cause mortality[46,47]; the effect seems to be specific to diarrhoea.

It would be highly desirable to know how Zn works. Is it an effect on immunity? There are two lines of evidence that suggest that Zn deficiency adversely affects immune function and that supplementation improves it. First, in human subjects there are data from the 1970s that, although not conclusive, support this contention. Children with acrodermatitis enteropathica, a congenital defect of Zn absorption, have thymic atrophy, lymphopenia, reduced lymphocyte response to mitogens, reduced delayed-type hypersensitivity and reduced immunoglobulin responses[48]. Many other reports of immune defects in patients with Zn deficiency are difficult to interpret because of comorbid processes (e.g. renal failure) that could themselves impair immunity. However, an important study in Indian children with diarrhoea has shown that Zn supplementation increases numbers of circulating CD3 and CD4 cells, but not CD8 cells, B-cells or natural killer cells[49]. In terms of innate immunity Paneth cells, which synthesise antimicrobial molecules for innate defence of the small intestine in human subjects, are also dependent on Zn[50,51]. Second, animal work supports this hypothesis. Zn deprivation of mice for a period as short as 30 d reduces cell-mediated immunity, delayed-type hypersensitivity, anti-tumour immunity and antibody responses by ≤80%[52]. Challenging Zn-deficient animals with low doses of *Trypanosoma cruzi* or intestinal nematodes results in death[53]. The deficiency state is associated with reduced numbers of lymphocytes as

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a result of impaired lymphopoiesis, but the production of antibody by each cell is not impaired. Furthermore, while Zn deficiency has marked effects on lymphoid cells, there is no effect on myeloid cells. It has been suggested that maintenance of lymphocyte populations is very expensive in terms of Zn and other nutrients, and that in the face of nutritional stress innate defence is maintained at the expense of adaptive immune responses[52]. However, there is also evidence that innate immunity (natural killer cell function and phagocytosis by macrophages) is impaired in Zn deficiency and this effect may be a consequence of reduced oxidative burst capacity, e.g. in trypanosomiasis[54]. Zn itself induces release of IL-1, IL-6, TNFα and interferon-γ in macrophages but not T-cells and high supraphysiological concentrations suppress T-cell functions[55]. Early data suggest that Zn is important for maintenance of antimicrobial peptide delivery in the small intestine[51,56]. The most definitive evidence that Zn deficiency impacts on immune function in human subjects comes from experimental Zn deficiency induced by dietary restriction in human volunteers[57]. Deficiency reduces thymulin levels in blood and reduces CD4:CD8. Zn deficiency also reduces synthesis of the T-helper 1 cytokines IL-2 and interferon-γ, but not the T-helper 2 cytokines IL-4, IL-6 and IL-10. Natural killer cell activity is also reduced in the volunteers on a Zn-deficient diet. Clearly, much work remains to be done in this area.

Selenium

A well-designed study has demonstrated that Se supplementation improves responses to oral vaccines, providing direct evidence of a micronutrient effect on intestinal defence[58]. Twenty-two volunteers with low plasma Se concentrations were given modest doses of a Se supplement (≤100 μg/d) or placebo and then were challenged with oral polio vaccine and immune responses to the vaccine determined. Volunteers supplemented with Se were found to show increased T-cell proliferation and higher interferon-γ and IL-10 production by T-cells 7 d after vaccination, as well as more rapid clearance of the virus in faeces. Little is known about the impact of Se on intestinal defence.

Other antioxidants

In the elderly vitamin E supplementation for 4 months increases delayed-type hypersensitivity responses and increases antibody titres to clinically-relevant vaccines (hepatitis B, tetanus) but not Ig levels or T- or B-cell numbers[59]. Again, there are few data relating to intestinal infectious disease, but there is scant evidence that antioxidant supplementation reduces all-cause mortality (including from other infectious diseases) or gastrointestinal cancer[60,61].

Iron

Studies in human subjects with Fe deficiency have shown that Fe deficiency is associated with defects in both adaptive and innate immunity that are reversible with Fe therapy[62]. Adaptive immune defects include reductions in T-cell numbers, T-cell proliferation, IL-2 production by T-cells, production of migration inhibitory factor by macrophages and tuberculin skin reactivity. Innate immune defects include reduced neutrophil killing, probably a result of reduced myeloperoxidase activity and impaired natural killer cell activity.

However, overcoming these defects with Fe supplementation is far from simple. Lactoferrin in human milk chelates Fe and inhibits bacterial proliferation by depriving the bacteria of an essential nutrient. The bacteriostatic effect of human milk is abolished by Fe therapy, so that Fe therapy would be expected to increase neonatal intestinal infectious disease. In milk-drinking nomads Fe therapy is associated with an increase in *Entamoeba histolytica* infection[63], possibly as a result of saturation of the milk transferrin, which overcomes the protective effect. There are few other data on intestinal defence, but Fe supplementation increases risk of malaria[64] and it has been suggested that Fe-supplementation programmes need to take into account the important differences in risk and benefit between malarious and non-malarious regions[62].

Vitamin D

While there has been great interest in the recognition that vitamin D can modulate immune responses to *Mycobacterium tuberculosis*[65,66] and can up regulate expression of the antimicrobial peptide LL-37[67], there is as yet no evidence that this outcome impacts on intestinal defence.

Host defence influences the microbiome

There is very little evidence in human subjects for this contention. While it is certainly an important question, it has only recently become possible to address it; two recent important experiments have demonstrated that host defence can modulate the microbiome.

It has been shown that a transgenic mouse that expresses a human intestinal α-defensin 5 (in addition to its own complement of antimicrobial peptides) is protected against salmonellosis[68]. Furthermore, the composition of the flora is altered[69] and it is tempting to speculate that this outcome may be an important role of intestinal antimicrobial peptide expression, as it has been established that such expression responds to the microbiota[23].

Very interesting recent data from an *in vitro* model of oral plaque biofilm formation indicates that antimicrobial molecules can influence the relative abundance of many species in the buccal flora (D Devine, personal communication).

Can this interaction be manipulated? The case for and against probiotics

It would be useful if the susceptibility of individuals or populations against intestinal infection could be reduced by up regulating host defence or if the metabolome could be manipulated. However, very few instances have been demonstrated.

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Probiotics are orally-administered cultures of viable bacteria, which are claimed to re-populate the microbiota. Prebiotics are intended to modify the microbiota through provision of a balance of nutrients that favours the expansion of one bacterial group. Probiotics have been demonstrated to be efficacious in the prevention of childhood diarrhoea, which by implication means successful manipulation of some element of this interaction[70]. However, of all the trials included in the review only two were conducted in tropical populations who most need the interventions. Nevertheless, this efficacy of probiotics demonstrates the principle that manipulation is possible. A full discussion of the large literature relating to probiotics is outside the scope of the present article but some recent data are worth noting. In a cross-over trial a probiotic preparation has been shown to modify the composition of the flora, but the same effect is obtained with a heat-killed control, suggesting that its true effect might have been prebiotic rather than probiotic in nature[71]. The likely effect of probiotic preparations may actually be prebiotic, rather than the commonly supposed effect, for two reasons. First, prebiotics can undoubtedly manipulate the microbiota to good effect, such as when oral rehydration therapy containing resistant starch is used to shorten childhood diarrhoea[72]. Second, it is not immediately apparent that a dose of 10^{10} colony-forming units of a probiotic (many of which will be killed by gastric acid) could greatly influence a microbiota that comprises $\geq 10^{15}$ bacteria. More work is needed on that point.

Can this interaction be influenced? The evidence relating to nutritional modification

Nutritional manipulation of intestinal defence clearly works; Zn is without question an important element of treatment of diarrhoeal disease and vitamin A has an important role to play in prevention. However, their mechanism of action is not known; for vitamin A, even the metabolic fate of mega-dose supplements is unknown. There is a strong rationale for giving multiple micronutrients together when attempting to achieve physiological nutritional restitution, as free-living organisms do not consume single nutrients to excess (or very rarely). This situation is different from using single nutrients as pharmacological modulators of specific physiological processes. In relation to Zn supplementation during diarrhoea, it is not clear whether the dose given represents physiological restitution at a time when requirements are increased or whether the dose is supraphysiological and in fact a pharmacological manipulation of, for example, defensin expression. Future studies should try and dissect out which of these processes is in operation in each circumstance.

Of the different elements of intestinal defence that might mediate these nutrient effects, effects on T-cells and antimicrobial peptides seem to be the strongest candidates. A placebo-controlled trial in healthy volunteers in The Netherlands has found that a combined supplement of vitamins A, C and E with Zn increases delayed-type hypersensitivity responses but not lymphocyte subsets, oxidative burst or responses to tetanus toxoid vaccine[73]. As part of a recent randomised controlled trial of a daily multiple micronutrient supplement in Zambian adults[74] an analysis was also carried out of antimicrobial peptide expression and an up-regulation of the α-defensin HD5 was observed in malnourished (BMI $<18{\cdot}5\,kg/m^2$) individuals (P Kelly and T Shawa, unpublished results). Effects of micronutrients on T-cell and antibody responses at rest, during intestinal infection or following vaccination remain largely unexplored.

Concluding remarks

Obesity is emerging as a public health crisis that will shape the health challenges of the 21st century, yet one billion individuals are undernourished and often these conditions coexist in the same populations[75]. While this position is driven by inequality in an unjust world, the key to understanding these disorders may lie in the human metabolome[75], the microscopic and physiological mechanism through which human behaviours drive some of the burden of disease. Understanding the human metabolome may hold the key to colo-rectal cancer, to inflammatory disorders and to effective treatments for diarrhoeal disease (such as oral rehydration therapy improved with resistant starch[72]). The benefits of incisive research into the microbiome and its effects on physiology could be very important indeed.

Acknowledgements

P. K. is funded by the Wellcome Trust (grant no. 067948). The author declares no conflict of interest.

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