Feeding immunity: skepticism, delicacies and delights

Marc Veldhoen & Henrique Veiga-Fernandes

Immunologists studying the relationship between nutrition and immunological function face many challenges. We discuss here some of the historical skepticism with which nutritional research has often been faced and the complexities that need to be overcome in order to provide meaningful mechanistic insights.

A dread disease its rankling horrors shed,
And Death's dire ravage through mine army spread.
Never mine eyes such dreary sight beheld,
Ghastly the mouth and gums enormous swelled;
And instant, putrid like a dead man's wound,
Poisoned with fetid steams the air around.
No sage physician's ever-watchful zeal,
No skilful surgeon's gentle hand to heal,
Were found; each dreary mournful hour we gave
Some brave companion to a foreign grave.
—Luís de Camões, The Lusiads (1572)

In 1497 the explorer Vasco da Gama departed from Portugal to find a sailing route to India and the East, but at a devastating cost; of 170 crew members, only 54 survived the odyssey, with more than one third dying of scurvy. This nutritional disease results from a deficiency in dietary vitamin C, which causes the dramatic symptoms described by Luís de Camões in the epic poem *The Lusiads*. Strikingly, when Vasco da Gama's crew arrived at Mombasa, Kenya, the local king offered them oranges and lemons, clearing up the "disease that started in the mouth." Gama's recordings of a treatment for scurvy by means of nutrition were seemingly ignored by subsequent naval expeditions; even two centuries later, when Lord Anson left England for the South Pacific in 1740, scurvy took the life of 626 of his 961 crew members. It was only in 1753 that a randomized controlled trial (albeit extremely small) by James Lind reconfirmed that the consumption of fresh citrus fruits prevented scurvy[1]. The problem of scurvy had effectively been solved, yet this treatment made no sense to physicians. They were convinced that the disease, and all others, was caused by bad air or 'humoral imbalances', and under their influence ships continued sailing without fresh fruits on board. In Lind's *Treatise of the Scurvy*, the theory of treating the disease is based nearly exclusively on the balances of the humors; readers at the time saw nothing decisive, and Lind himself lost faith in his own remedy when employing heat to concentrate lemon juice and rejected the claim in the third edition of his treatise that "abstinence from fresh vegetable food and greens is alone the true primary cause of the disease"[2]. Today, malnutrition and its consequent increased susceptibility to infections remain of major importance. However, the steep rise in obesity, autoimmunity and allergic disorders, coinciding with a different style of diet, have emphasized the requirement for a much more in-depth and mechanistic knowledge base on nutrition and immunity.

Marc Veldhoen is with the Laboratory of Lymphocyte Signalling and Development, The Babraham Institute, Cambridge, UK. Henrique Veiga-Fernandes is with the Instituto de Medicina Molecular, Faculdade de Medicina de Lisboa, Lisboa, Portugal.
e-mail: marc.veldhoen@babraham.ac.uk or jhfernandes@medicina.ulisboa.pt

Early observations and successes

Observing chickens at the end of the nineteenth century in the Dutch East Indies (Indonesia), the Dutch physicians and physiologists Christiaan Eijkman and Adolphe Guillaume Vorderman discovered that the diet needs to include a complex essential compound, now known as vitamin B_1, to prevent beriberi. Additional work by Gerrit Grijns, the English biochemist Frederick Gowland Hopkins (who also discovered the amino acid tryptophan) and the Polish biochemist Casimir Funk resulted in the term 'vital amines' (or 'vitamins').

The history of the discovery of complex micronutrients vital for life is full of serendipity and Nobel prizes in physiology or medicine and in chemistry. An amazing 17 Nobel prizes have been awarded for vitamins, with vitamin B_{12} topping the list, winning the prestigious prize no less than four times (1934, 1957, 1964 and 1965). The discovery of vitamin B_{12} started with the observation that the consumption of raw liver could cure patients of pernicious anemia, but the compound responsible for this effect remained obscure[3]. The Nobel committee seemed to be reluctant to acknowledge the importance of food-based research and compounds, possibly owing to the many skeptics who proclaimed vitamins to be only hypothetical entities that seemed to cure a (too) large number of phenomena. Only after the Dutch scientists Willem Donath and Barend Jansen obtained pure vitamin B_1 and showed, in 1926, that as little as 10 μg a day was sufficient to prevent beriberi were sufficient numbers of skeptics convinced.

Early skepticism

Distinct micronutrients often coexist in the same or similar foods, which confounded pioneering attempts to delineate the nutritional causative agent, especially at a time when biochemistry was a very young field of science. In addition, many observations

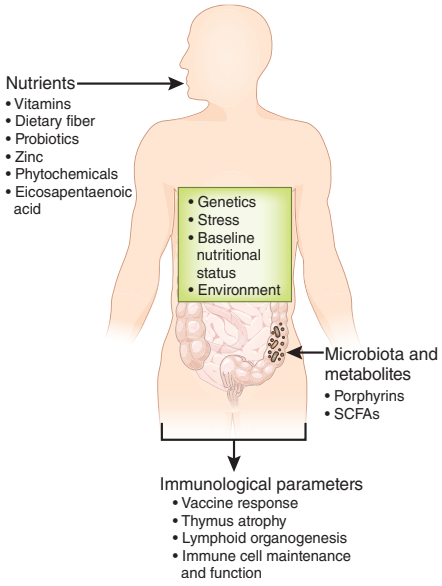

Figure 1 Nutrition is an important determinant of immunity. From the nineteenth century, clinical and epidemiological studies have highlighted the potential importance of micronutrients in immunocompetence. Examples of some important micronutrients are shown here together with the host factors, including the microbiota, that influence their action on immunity. SCFA, short-chain fatty acids.

potentially linking micronutrients to disease were made in the same era as the great discoveries of the microbial causes of disease by Robert Koch and Louis Pasteur. Their work produced explanations for sickness that captured the public's imagination, especially with the focus on common infectious diseases such as smallpox, anthrax and tuberculosis. Moreover, many observations made in the nineteenth century about diseases now known to be caused by micronutrient deficiencies, such as beriberi, pellagra and xerophthalmia, were originally assumed to be the result of pathogens or toxins. Patients who had nutrient deficiencies also had infections, and many fruitless attempts were made to isolate and identify the microorganisms responsible.

Interestingly, recent years have seen a dramatic rise in the understanding of how microorganisms make crucial contributions to human health, shaping the immune system and metabolic pathways—notably those involved in micronutrient synthesis—not present in the mammalian host. In-depth reviews of the role of the microbiota and the potential benefits of pro- and prebiotics have been discussed elsewhere[4] and will not be part of this Commentary.

Linking nutrients and immunity

The earliest documented examples of micronutrients' influencing immunity date from the nineteenth century. Such work indicated previously unappreciated links with immunity and made a strong case that infections did not cause observed disorders and pathologies but were secondary to deficiency in micronutrients. Supported by clinical and epidemiological studies, which show a substantial negative correlation of nutritional deficiencies with immunocompetence and the reverse correlation with the risk of infection and related pathologies, is the idea that nutrition is an important determinant of immunity is now generally well accepted (**Fig. 1**). Nevertheless, the impact of dietary elements in immune system–related processes is still often dismissed, even when substantial amounts of data correlate diet with immune system–related related disorders.

Early scientific data correlated cachexia to impaired immunocompetence, linking malnutrition to lymphoid tissue atrophy. In 1810 J.F. Menkel noted that the human thymus, although its importance as the organ in which T cells develop was not understood at the time, was strongly affected by malnutrition[5]. By 1845 John Simon recorded that the thymus is "a barometer of malnutrition, and a very sensitive one"[6] and 'nutritional thymectomy' became a medical term in vogue. Interestingly, subsequent epidemiological studies have correlated thymic size in infants to maternal nutrition during pregnancy, and although several attempts have been made to identify the nutritional compounds responsible for such drastic effects, their exact nature and their mechanism of action remain controversial[7].

There is an emergent interest in understanding how the 'Western diet' affects immunity. Many people across the globe have adopted this diet, and epidemiological studies have revealed that it correlates with a high incidence of chronic inflammatory disorders, including diabetes, multiple sclerosis and asthma[4]. Nevertheless, because the Western diet includes a large proportion of red meat, sugars, fats and refined carbohydrates and relatively small amounts of vegetables, fruits and fish, it is likely that the causative component of its associated pathologies is not a single entity but a complex array of unbalanced abundance of micronutrients in the diet.

However, epidemiological studies have highlighted roles for specific micronutrients in immunity. For example, mounting evidence[8,9] correlates vitamin A deficiency, which is still highly prevalent among children and pregnant women in several African and Asian countries[10,11], with a high risk of intestinal and respiratory infections. These observations were substantiated by experimental data as early as 1925, when rats on a diet deficient in vitamin A were found to develop thymic atrophy[12]. It is now understood that different processes require different amounts of micronutrients, with the immune system generally the largest consumer. In view of this, the World Health Organization established a vitamin A–supplementation program, which had a positive effect on morbidity and mortality rates of infectious diseases in deprived children[13,14]. Interestingly, low vitamin A levels are also associated with reduced efficacy of vaccination protocols[15], but the benefits of vitamin A supplementation during vaccination are still a matter of controversy[16–18].

Delicacies under scrutiny

There is little doubt that defined micronutrients can prevent and cure nutritional illnesses. However, news headlines make strong and often contradictory statements on the role of nutrients, from advising people to drink red wine and consume 'superfoods' to warning against the dangers of fats and sugars. Health advice fluctuates over time and generations, and debates on the benefits (or lack thereof) of organic foods and genetically modified foods have contributed to confusion and skepticism by the general public, policymakers and scientists. Scientific scrutiny of the original research that generates the headlines is often minor, if present at all, and the results of that research are often derived from preliminary, small-scale studies or from conference communications that have not undergone peer review. Moreover, commercial and funding interests can be intertwined with the results. Hence, the use of food supplements continues to raise eyebrows among the scientific and medical communities and has even fueled resistance to clinical trials and basic research around 'nutrition' therapies. The public is left to ponder which dietary elements contribute to health and what to do when told only in very general terms that some foods are 'good' and 'enhance immunity'.

The large and varied number of observations made about micronutrient deficiencies, and the small amounts of those micronutrients that are required, has probably contributed to the skepticism about the existence and importance of these micronutrients, as they may seem reminiscent of the snake oil and wonder potions proclaimed to cure nearly every affliction. Many micronutrients have important roles in cell metabolism and affect different cell types and processes to different degrees. Furthermore, many micronutrients are not themselves metabolized but instead act as classical catalysts, and are recycled many times over. As a result, some

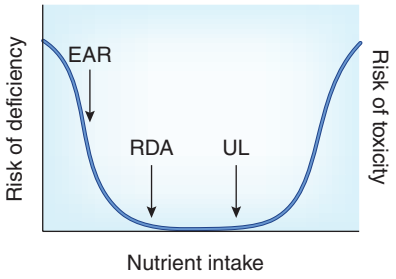

Figure 2 Relationship between nutrient intake and health risk. Plotting of nutrient consumption versus risk of harm caused by nutrient deficiency and toxicity generates a U-shaped curve. EAR, estimated average requirement; RDA, recommended dietary allowance; UL, tolerable upper level. Adapted from ref. 38. Reprinted with permission from the National Academies Press, Copyright 2006, National Academy of Sciences.

consequences of a deficiency may not become apparent in a person, much like a genetic disorder 'skipping' a generation, and may hence be misdiagnosed. A good example is vitamin B_{12}; its dietary deficiency in mice does not result in its absence in the serum of the first generation. Sufficient amounts for essential processes are passed from the mother to its progeny, but the deficiency does have a considerable influence on some health aspects in the first generation. It is the second generation of mice that is more dramatically affected by the consequences of vitamin B_{12} deficiency but can be fully 'rescued' by a single administration of 200 μg of this micronutrient[19].

Data from double-blinded, placebo-controlled clinical trials have suggested that nutritional supplements have intangible consequences on the health of human beings from developed countries. However, it has been argued that the lack of scientific evidence for supplement use may result from poor experimental design, data analysis or unclear definitions of baselines for a given nutrient. A clear example has been provided by the unexpected results of a vaccination trial with vitamin A in children from Ghana. Participants who received high doses (50,000 international units) of vitamin A at vaccination priming had higher mortality rates over a 6-month period after vaccination than did participants given a placebo[20]. Similarly, in a cancer-prevention trial with vitamin A in subjects who smoked, participants who received high doses of β-carotene for 5–8 years had an 18% higher chance of developing lung cancer than did those who received a placebo[21]. Overall, these disappointing results indicate that a nutrient may have beneficial effects at low doses but negative or toxic effects at higher doses. Thus, nutrient risk curves are J- or U-shaped, but a person's response to a given nutrient will always depend on his or her baseline at the start of the trial (**Fig. 2**).

Confounding factors such as those outlined above have hampered diet trials and have engendered long-lasting skepticism about nutritional prevention or treatment strategies. Thus, in 2014, a set of guidelines to apply in nutritional trials was proposed[22]. The guidelines include assessment of the dose-response curve of a given nutrient, the use of participants with similar baselines of nutrient intake, the use of rigorous methodology to define quantities of food and nutrient intake and the exclusion of confounding factors[22] (**Table 1**).

Mechanism, mechanism, mechanism

The immune system is complex in its array of cell types and their functions, but is also highly integrated in all body systems. Beyond their classic role in fighting pathogens and pathogen products, cells of the immune system play important parts in tissue homeostasis. Furthermore, immunological functions are not restricted to cells of the immune system but are an integral part of most cell types. Immunological functions are always active, but they are called upon especially in times of tissue damage and the presence of invading microorganisms. Immune system activity is metabolically demanding, requiring a greater presence of metabolites and substrates. Hence, epidemiological studies may not always establish a direct link between nutritional deficiencies and classic immunological functions. In addition, the effects of nutritional deficiencies may become apparent only upon challenge of an organism, such as an infection or vaccination, during stress on a particular tissue or when confounding environmental or genetic factors are at play. The highly integrated nature of immunity requires that specific questions be addressed in particular physiological or pathological states in relation to nutritional requirements.

Despite the health benefits and potential of micronutrients, fundamental mechanisms linking particular nutrients with physiological consequences are, in many cases, undetermined. Mechanistic understanding and well-documented scientific data on the relationships between nutrients and health will greatly advance the development of food products and nutritional guidelines and offer potential low-cost, efficient intervention strategies. Without basic science and mechanistic insights, any guidelines and health claims will remain compromised, open to speculation and of substantial concern to scientists and decision-makers. Reliable scientific evidence underpins accurate nutritional advice and effective public-health policies that determine the optimal consumption of macro- and micronutrients in health and disease. Currently, even at the population-wide level, the optimal intake of many nutritional components is uncertain and is based on limited data, with undetermined baseline ranges for physiological concentrations. Nevertheless, some molecular pathways have been studied in sufficient detail to reveal nutritional sensing strategies that shape and define the fate of cells of the immune system and disease outcomes. Some amino acids, fats (such as omega-3 fatty acids) and vitamins (notably vitamin A and vitamin D) can be included in this category.

As an example, genetically modified mice with a hematopoietic cell–autonomous deficiency in retinoic-acid (RA) signaling (RA

Table 1 Nutritional clinical trial and meta-analysis guidelines

Nutritional clinical trial guidelines	Guidelines for inclusion of studies in meta-analyses
Basal nutrient status must be used as an inclusion criterion in the trial.	All studies included must have started from similar nutrient baselines and have followed the guidelines for individual clinical trials.
Nutritional intervention must be large enough to change nutrient status while allowing suitable quantification analyses.	Studies must use similar doses.
Change in the nutrient status of those enrolled in trials must be measured and recorded.	All studies included must have used the same chemical form of the nutrient and the same food matrix.
The hypothesis to be tested must be that a change in nutrient status (not just in the diet) produces the sought-after effect.	All studies included must have the same conutrient status.
Conutrient status must be monitored to exclude other confounding limiting factors in the response.	All studies included must have had approximately equal periods of exposure to the altered nutritional intake.

Source: ref. 22.

Chris Sharp/Nature Publishing Group

For centuries sailors suffered from scurvy, a result of vitamin C deficiency, but the evidence of this causative relationship was ignored. This is but one example of the historical skepticism that nutritional research has faced.

is a vitamin A metabolite), have impaired differentiation of fetal group 3 innate lymphoid (ILC3) cells. ILC3 cells are essential in determining the development of secondary immune organs, and RA directly regulates the master ILC3 cell transcription factor RORγt[23]. Importantly, the lack of RA signaling in ILC3 cells *in utero* results in irreversibly small lymphoid organs in adulthood, which severely compromises immune responses to pathogens[23]. Vitamin A deficiency has also been shown to shift immune responses from adult ILC3 cells, associated with antibacterial activity of type 3 immunity, to antiworm and tissue-repair (ILC2) responses[24]. Furthermore, vitamin A can modulate expression of the gut-homing receptors $\alpha_4\beta_7$ and CCR9 in conventional T cells[25] to enhance the induction of regulatory T cells, inhibit effector T cell responses and control the expression of effector molecules from lymphoid cells[26,27]. RA also has a direct immunoglobulin A–promoting effect on activated B cells in the gut[28].

Considerable progress has been also made in understanding the role of some phytochemicals in cells of the immune system, notably through the elucidation of aryl hydrocarbon receptor (AhR). This molecule belongs to a highly conserved family of basic helix-loop-helix transcription factors that act as sensory proteins, mediating responses to environmental factors such as oxygen pressures and light-dark cycles. Interestingly, AhR ligands in the mouse intestinal lumen can originate from cruciferous vegetables that produce the phytochemical indole-3-carbinol, which, through oxidation and chemical condensation in the stomach, is converted into a high-affinity AhR ligand[29]. Strikingly, AhR activity is an important regulator of the maintenance of intraepithelial lymphocytes, tissue-resident memory cells and the proliferation of adult ILC3 cells[30–32], which establishes a direct link between dietary phytochemicals and cells of the immune system required for mucosal homeostasis and defense.

Various genes, gene products and increasingly smaller subsets of cells can now be studied with the technological advances of the past few decades. This has enabled much more refined experimentation. Next-generation sequencing techniques make it possible to obtain a more holistic picture of the effects of micronutrients on cellular processes. Similarly, advances in multiparameter flow cytometry and imaging, mass spectrometry and genetic manipulations have provided novel and essential tools for addressing the role of micronutrients at the level of the organism, tissues and individual cells and genes. New technology platforms offer unprecedented opportunities to delineate nutritional pathways, but the pleiotropic effects of most micronutrients and the requirement for tissue-specific and/or inducible systems to define any 'nutritional' molecular axis in cells of the immune system has hampered progress in the field.

Confounding factors

It is important to accurately define the nutritional needs throughout the life stages and recognize that there are different nutritional requirements during development, in old age and during infectious challenges and chronic inflammation. In addition, events in early life, such as reduced access to micronutrients, can have a substantial effect on later life and the capacity to mount appropriate immune responses[23]. Malnutrition is the most common cause of immunodeficiency worldwide, and the impaired immunocompetence that results from it may exist for many years. In addition, and for various reasons, older subjects tend to have a higher prevalence of nutrient deficiencies. This highlights the importance of determining baseline requirements and past histories in human studies, already confounded by the genetic variance among the human population. Furthermore, and as mentioned above, activation of the immune system requires a large amount of nutrients to fuel multiple metabolic pathways. The relationships among food, nutrition and health are complex, and disentangling them will require integration of data from diverse research communities.

Opening the box of delights

Components of living organisms or natural products, now widely used in the clinic, can be part of the human diet and include panoplies of pharmaceutical compounds, ranging from antibiotics to anticancer agents or immunosuppressors. Over the past decades, much of the research into natural products has been focused on exotic agents derived from soil and marine bacteria, but the field has awakened to recent discoveries showing that the human body contains symbiotic microbial communities that are copious producers of as-yet-unknown natural products[33,34]. This is a research field that is growing to maturity, evolving to proceed down unknown roads, questioning itself but paving the way as it progresses.

An area long associated negatively with health is dietary fat. However, mammals are unable to generate essential fatty acids (eicosapentaenoic acids) and need to obtain them via the diet, as is true of many micronutrients. Among these essential fatty acids are the omega-3 fatty acids, which are found in nuts, some fruits and vegetables, and fish. Their role in immunity has been established, but many questions remain about their mode of action and cellular targets[29]. In contrast, dietary fiber has long been associated with health. However, the molecular mechanisms of its actions have remained obscure. Fibers are metabolized and broken down into short-chain fatty acids such as acetate and butyrate. The latter has been demonstrated to be of importance not only in maintaining the health of colonocytes but also in immunolotolerance in the intestine as well as in peripheral organs[35–37]. The identification of several receptors for short-chain fatty acids and their genetic deletion has contributed to much better and more-detailed understanding of their roles in health. Additional micronutrients remain to be identified and linked with the function of cells of the immune system. Among these are phytochemicals, some of which have a yet unknown identity, and functional amino acids, such as methionine, with a known influence on general health and lifespan but with largely unknown effects on immunity.

Where does 'nutritional immunology' stand? The historical collection of casuistic discoveries, epidemiology studies and more recent mechanistic work indicates that the challenges ahead lie in defining the molecular links between nutrients and cells of the immune system that form the basis of protective immunity. Novel high-throughput technology platforms offer real opportunities to shed light on new nutritional delights

for the immune system. However, although opportunities are there for the taking, this will occur only if the research community is alert and open to the ever-growing evidence that nutrients shape and define immunity.

ACKNOWLEDGMENTS

We thank M. Ferreira for discussions. Supported by the UK Biotechnology and Biological Sciences Research Council Institute (Strategic Programme grant to M.V.), the European Research Council (H.V.F.; and 280307 to M.V.), Fundação para a Ciência e Tecnologia, Portugal (H.V.F.), the Kenneth Rainnin Foundation (H.V.F.) and the Crohn's and Colitis Foundation of America (H.V.F.).

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

1. Martini, E. *Lancet* **361**, 1480 (2003).
2. Lind, J. *Treatise on the Scurvy*, 3rd ed. (1772).
3. Chanarin, I. *Br. J. Haematol.* **111**, 407–415 (2000).
4. Maslowski, K.M. & Mackay, C.R. *Nat. Immunol.* **12**, 5–9 (2011).
5. Beisel, W.R. *J. Nutr.* **122** (suppl.), 591–596 (1992).
6. Simon, J. *A Physiological Essay on the Thymus Gland.* (H. Renshaw, London, 1845).
7. Ferreira, M. & Veiga-Fernandes, H. *BioEssays* **36**, 1213–1220 (2014).
8. Sommer, A., Katz, J. & Tarwotjo, I. *Am. J. Clin. Nutr.* **40**, 1090–1095 (1984).
9. Black, R.E. *et al. Lancet* **382**, 427–451 (2013).
10. West, K.P. Jr. *J. Nutr.* **132**, 2857S–2866S (2002).
11. World Health Organization. Global prevalence of vitamin A deficiency in populations at risk 1995–2005. in *WHO Global Database on Vitamin A Deficiency* (World Health Organization, Geneva, 2009).
12. Wolbach, S.B. & Howe, P.R. *J. Exp. Med.* **42**, 753–777 (1925).
13. Diness, B.R. *et al. Br. J. Nutr.* **105**, 1819–1822 (2011).
14. Rahmathullah, L. *et al. Br. Med. J.* **327**, 254 (2003).
15. Kaufman, D.R. *et al. J. Immunol.* **187**, 1877–1883 (2011).
16. Benn, C.S., Fisker, A.B., Jorgensen, M.J. & Aaby, P. *Vaccine* **25**, 777–779 (2007).
17. Benn, C.S. *et al. Br. Med. J.* **331**, 1428–1432 (2005).
18. Vlasova, A.N. *et al. J. Immunol.* **190**, 4742–4753 (2013).
19. Roman-Garcia, P. *et al. J. Clin. Invest.* **124**, 2988–3002 (2014).
20. Newton, S. *et al. J. Nutr.* **135**, 2669–2673 (2005).
21. The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group. *N. Engl. J. Med.* **330**, 1029–1035 (1994).
22. Heaney, R.P. *Nutr. Rev.* **72**, 48–54 (2014).
23. van de Pavert, S.A. *et al. Nature* **508**, 123–127 (2014).
24. Spencer, S.P. *et al. Science* **343**, 432–437 (2014).
25. Iwata, M. *Semin. Immunol.* **21**, 8–13 (2009).
26. Hall, J.A. *et al. Immunity* **34**, 435–447 (2011).
27. Mucida, D. *et al. Immunity* **30**, 471–472, author reply 472–473 (2009).
28. Mora, J.R. *Semin. Immunol.* **21**, 28–35 (2009).
29. Veldhoen, M. & Brucklacher-Waldert, V. *Nat. Rev. Immunol.* **12**, 696–708 (2012).
30. Kiss, E.A. *et al. Science* **334**, 1561–1565 (2011).
31. Li, Y. *et al. Cell* **147**, 629–640 (2011).
32. Zaid, A. *et al. Proc. Natl. Acad. Sci. USA* **111**, 5307–5312 (2014).
33. Cimermancic, P. *et al. Cell* **158**, 412–421 (2014).
34. Donia, M.S. *et al. Cell* **158**, 1402–1414 (2014).
35. Arpaia, N. *et al. Nature* **504**, 451–455 (2013).
36. Furusawa, Y. *et al. Nature* **504**, 446–450 (2013).
37. Singh, N. *et al. Immunity* **40**, 128–139 (2014).
38. Otten, J.J., Hellwig, J.P. & Meyers, L.D. (eds.) *Dietary Reference Intakes: The Essential Guide to Nutrient Requirements* (National Research Council, National Academies Press, Washington DC, 2006).