

Food and Nutrition Open Access

ISSN 2517-5726

Crucifixion with a Donor: Sponsored Research on the Effects of Food and Nutrition on the Immune System

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Introduction

At the time of Jheronimus Bosch, one of the greatest painters of The Netherlands who lived from around 1450 to 1516, it was not unusual that the donor who commissions a painting and therefore also had to pay for it, was included in the painting (Figure 1). In the Crucifixion with a donor, depicting the Calvary scene with Jesus Christ on the cross, all personages have an evident role, apart from the donor. The conflict of interest for Jheronimus Bosch is evident: not including the donor in the painting would have meant a no-go for the deal.

The dilemma between sponsorship and artistic freedom in art likewise applies to scientific integrity for sponsored research. Before discussing this theme in more detail, focused on food and nutrition research, first the relationship between food, nutrition and the immune system will be summarized.

Food and Nutrition influence the Development and Function of the Immune System

In order to carry out biological functions, a continuous supply of energy is necessary. To ascertain continuity, the (human) body has energy reserves that are activated during periods of fasting. These energy reserves are used in such a way that the most important body functions are given priority. A continuous supply of energy is also required for the proper functioning of the immune system [1,2].

Apart from the general energy requirements, certain dietary components specifically regulate and modulate the immune system. Deficiencies in macronutrients (proteins, carbohydrates, fats) and micronutrients (vitamins, minerals, trace elements) lead to a clinically significant secondary immunodeficiency with a strongly increased risk of infection. At the other end of the spectrum, in case of overeating and obesity, the immune system also gets out of balance and the risk of infection is similarly increased [3]. It is therefore important to study the effects of food components on the immune response.

Article Information

DOI: 10.31021/fnoa.20182111
Article Type: Opinion Article
Journal Type: Open Access
Volume: 2 **Issue:** 1
Manuscript ID: FNOA-2-111
Publisher: Boffin Access Limited

Received Date: 29 October 2018

Accepted Date: 13 November 2018

Published Date: 16 November 2018

*Corresponding author:

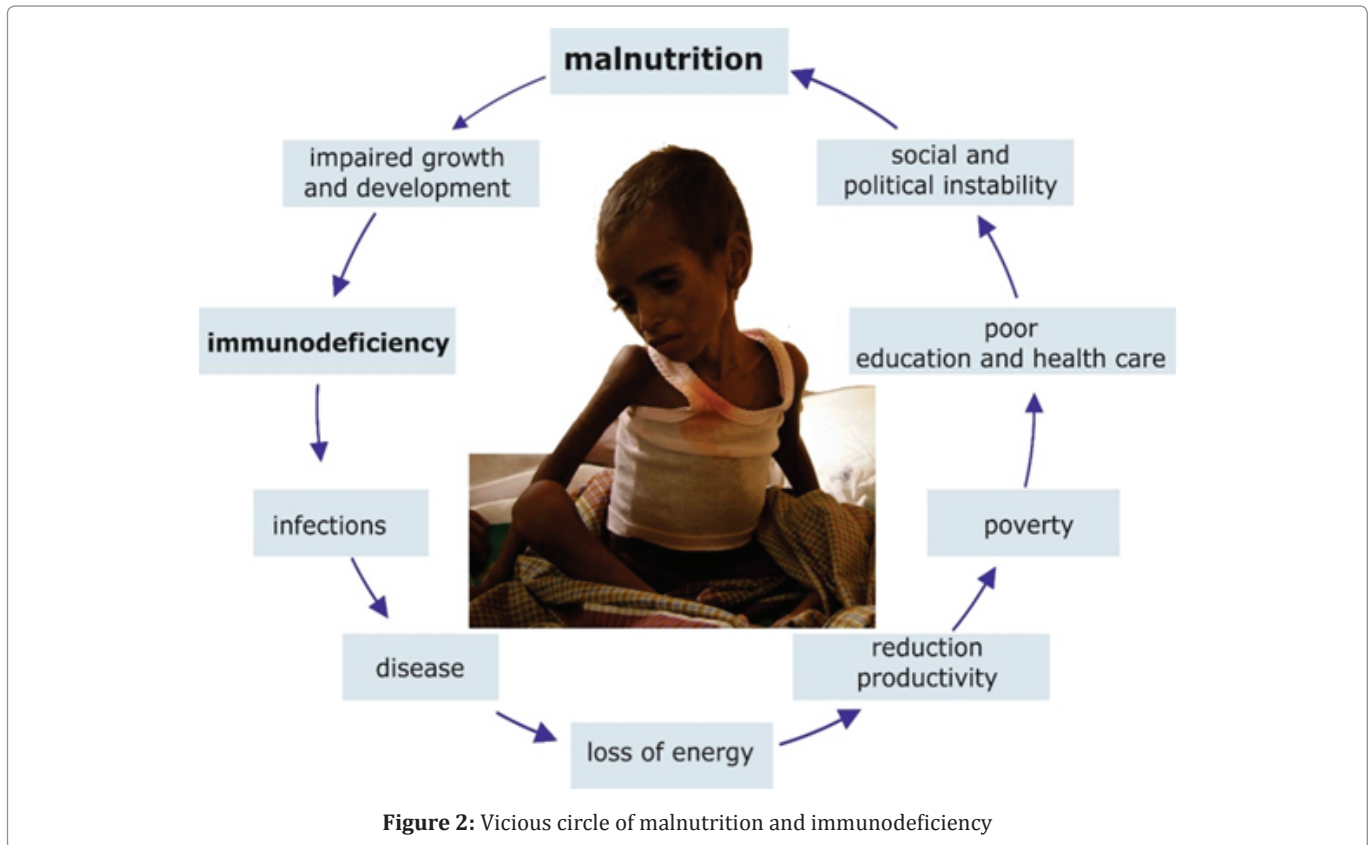
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Citation: Ger T. Rijkers. Crucifixion with a Donor: Sponsored Research on the Effects of Food and Nutrition on the Immune System. Food Nutr OA. 2018 Nov;2(1):111

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Figure 1: Detail of Crucifixion with a donor by Jheronimus Bosch, ca 1490. Royal Museums of Fine Arts of Belgium, Brussels. Source Wikimedia Commons https://commons.wikimedia.org/wiki/File:Hieronymus_Bosch_-_Crucifixion_with_a_Donor_-_WGA02494.jpg Accessed June 29, 2018



Total Malnutrition mainly causes a Greatly reduced Cellular Immunity and an Increased Risk of Infection

Malnutrition resulting from both a shortage of proteins and ingested calories (total malnutrition, protein caloric (energy) malnutrition) is perhaps the most important cause of reduced defense against infections worldwide. This problem mainly occurs in developing countries but also occurs in the western world, especially in the elderly and in patients in hospitals and nursing institutions. In developing countries, approximately 50 percent of all deaths caused by infectious diseases among children younger than 5 years are associated with malnutrition. Infectious diseases resulting from malnutrition have a major impact on the functioning of society as a whole and contributes to poverty [4,5] (Figure 2).

Severe total malnutrition in children leads to a disturbed development of the thymus [6]. As a result, the production of T-lymphocytes is greatly reduced, a T-lymphocytopenia develops, secondary lymphoid organs are not well developed and the cellular immune response is ultimately severely impaired [7]. It is striking that in total malnutrition, cellular immunity is more affected than humoral immunity: the serum concentrations of antibodies are usually normal (or even somewhat increased). In addition to impaired cellular immunity, in severe malnutrition, the cells of the innate immune system can also dysfunction (reduced killing after phagocytosis and reduced antigen presentation) and the mucous membranes are also affected [8]. Malnutrition also affects leptin levels. The hormone, produced by adipose tissue has immune modulating functions; it stimulates the secretion of pro-inflammatory cytokines and the Th1 response [9]. The clinical consequences of the effects of malnutrition on the functionality of the immune system are an increased frequency of bacterial, viral, parasitic and opportunistic infections, including tuberculosis and infections with *Pneumocystis jiroveci* [10-12].

Deficiencies in certain Micronutrients can lead to Specific Secondary Immune Deficiencies

In addition to total malnutrition, deficiencies in certain micronutrients (such as vitamins and minerals) can also lead to

secondary immunodeficiencies. In these cases, both the innate immune system and the acquired cellular and humoral immune system can be affected. After birth, breastfeeding provides an optimal, balanced and sufficient amount of proteins, fats, carbohydrates and micronutrients such as iron, zinc, selenium, vitamin A, polyunsaturated fatty acids (PUFA) and nucleotides for the baby and its developing immune system. After weaning, and depending on the diet, micronutrient deficiencies could occur with consequences for the immune system. Micronutrient deficiency may be part of an overall malnutrition state and worsen its consequences, in terms of infection sensitivity. Some common deficiencies will be briefly discussed here for illustrative purposes.

Zinc

A zinc deficiency (present in a third of the world's population) can be the result of insufficient intake of the mineral, of a disease (e.g. celiac disease, diarrhea), or can sometimes be based on a primary genetic defect (acrodermatitis enteropathica) [13,14]. The latter condition is the result of a mutation in a zinc transport protein. Zinc deficiency is common in newborns, young children and women and is partly responsible for lagging growth and cognitive development in children. Zinc is an important co-factor for the activity of many enzymes, including the hormone thymulin that is produced by epithelial cells in the thymus. Thymulin is important for the differentiation of T lymphocytes. Zinc deficiency leads, among other things, to thymic atrophy, reduced production of Th1 cytokines (but not Th2 cytokines) and a reduced function of NK cells, macrophages and granulocytes [15]. However, the dose-effect relationship is complex: in T-lymphocytes, activation occurs with normal amounts of zinc but inhibition with high concentrations, while macrophages become more active at higher concentrations. Supplementation with zinc leads to more protein and DNA synthesis and increased metabolic activity, but also a strong acute phase response and inflammation. Clinically, a zinc deficiency will express itself in increased infection sensitivity and diarrhea. In the case of acrodermatitis (enteropathica), acute dermatitis, alopecia and chronic diarrhea (Figure 3) are included as symptoms [14]. Zinc supplementation often relieves both the immunological abnormalities and the clinical symptoms [15].



Figure 3: Acrodermatitis enteropathica. The most dominant feature upon clinical examination in this case is an erythematous skin rash that can have an eczematous appearance. The symptoms start to occur after breastfeeding is stopped. The lesions occur perioral, perianal, on hands, feet and the hairy head. The skin can become secondary infected by *S. aureus* or *C. albicans*. In case of chronic zinc deficiency, chronic diarrhea and alopecia are also found. Administration of zinc in supra-physiological amounts causes all symptoms to disappear.

Iron

Like with zinc deficiency, iron deficiency can also lead to increased sensitivity for infections. Iron deficiency is associated with reduced phagocytic activity and intracellular killing by phagocytes, reduced NK cell function and reduced differentiation and proliferation of Th lymphocytes (Th1 lymphocytes are more sensitive than Th2 lymphocytes) [16]. Iron is also involved in the regulation of cytokine production and activity and induces more IFN- γ production with CD4+ T lymphocytes and TNF- α production with macrophages [17]. Although iron deficiency has negative effects on the immune system, excessive iron supplementation must also be avoided. The reason is that an iron overload can also result in an increased risk of infection, in particular, from microorganisms that iron for their growth (especially *Listeria* and *Yersinia* species) [18]. This occurs especially in children with malaria and malnutrition in developing countries.

Selenium

Selenium is essential for optimal immune responses and affects both the innate and the acquired immune system. It plays a role in the regulation of redox reactions and has an antioxidative function. It helps to protect the host against oxidative stress that occurs during the respiratory burst in phagocytes as a result of an infection or trauma [19]. Selenium deficiency has numerous effects, including reduced chemotaxis of neutrophilic granulocytes, reduced antibody production by B lymphocytes, reduced NK cell activity and an increase in the numbers of CD4+ T lymphocytes, but decreased numbers of CD8+ T lymphocytes. A lack of selenium leads to increased sensitivity to enteroviruses, especially coxsackievirus, and a faster progression to AIDS in HIV-infected patients [20,21].

Vitamin A

Vitamin A deficiency is prevalent worldwide (140 million young children) and has many consequences on the functioning of the immune system. It plays an important role in both innate and acquired immune system [22-24]. Vitamin A deficiency is associated with reduced hematopoiesis, reduced phagocytic activity, decreased respiratory burst in phagocytes, reduced NK cell number and function, increased production of IL-12 and TNF- α , reduced proliferation of lymphocytes, impaired development and differentiation of Th1 and Th2 lymphocytes and a reduced humoral immune response [25]. Vitamin A deficiency also leads to a reduced integrity of the mucosal barriers, especially due to the disappearance of mucus-producing goblet cells in the small intestine. The metabolite of vitamin A, retinoic acid, plays a role in the homing of activated T-lymphocytes

to mucosal tissues and subsequently the formation of Treg cells in the mucosa responsible for maintaining oral tolerance. Retinoic acid cooperates with TGF- β and is also involved in the formation of IgA plasma cells in the intestinal mucosa [26]. As a result of vitamin A deficiency, there is an increase in the occurrence of respiratory and intestinal infections, and a large number of these infections often lead to more serious consequences. Vitamin A supplementation is not very effective in well-fed populations, but certainly of great importance in risk populations such as children in developing countries [27].

Vitamin D

Deficiencies in vitamin D are associated with reduced function of the immune system and contribute to the development of immune mediated inflammatory diseases, such as type 1 diabetes, multiple sclerosis and rheumatoid arthritis [28,29]. Vitamin D binds to a specific vitamin D receptor (VDR) that can control the expression of genes that inhibit the immune response and expression of cytokines. The VDR occurs in CD4+ and CD8+ T lymphocytes, B lymphocytes, neutrophils and macrophages and dendritic cells, and the VDR expression is regulated by activation of T lymphocytes [30]. Exposure of T lymphocytes to 1,25-dihydroxyvitamin D results in a strongly increased expression of VDR and reduced secretion of IL-2 and IFN- γ (Th1 cells) and IL-17 and IL-21 (Th17 cells) with a simultaneous increase of IL-4 and IL-13 (Th2 cells). The latter is due to the induced expression of GATA-3 and c-maf transcription factors that stimulate the differentiation of Th2 cells. Vitamin D induces IL-10 producing antigen-specific regulatory Tr1 cells (CD4+ CD25+) that play a crucial role in tolerance induction. Monocytes and macrophages are stimulated by vitamin D to phagocytosis, production of IL-1 β and the antibacterial defensin β 2 and cathelicidins [30,31].

Vitamin E and C

Vitamin E and C are strong antioxidants. Vitamin E optimizes and strengthens the immune response by inhibiting prostaglandin production by macrophages, stimulating IL-2 production and inducing T-lymphocyte proliferation. It has, amongst other things, effects on lymphocyte proliferation, NK cell activity and phagocytosis. Vitamin E directs the immune response towards a Th1 response [32]. Deficiencies of vitamin E are rare, but in the elderly, supplementation of vitamin E has beneficial effects on the functioning of the immune system (especially the Th1 response) [33]. Vitamin C also is involved in many functions of the cells of the immune system, resulting in an enhanced immune response to infections [34]. Vitamin C is present in leukocytes in relatively high concentrations. It is a (negative) regulator of the inflammatory response and plays a role in chemotaxis, NK cell function, lymphocyte proliferation and bactericidal activity of phagocytes [35].

Prebiotics and Probiotics

The composition and functionality of the gut microbiota has a major impact for the development and function of the (mucosal) immune system. Therefore, microbiota management in the form of supplementation with prebiotics and/or probiotics is being used to restore and maintain a balanced immune system. Many excellent reviews have recently been published on this subject, which is why this type of food supplements is not discussed here [36-39]. The succeeding section on conflict of interest also applies fully to this field of research.

Scientific Integrity and Conflict of Interest

Most of the information cited above has been obtained from human intervention studies. These studies in general require large numbers of participants who will follow a specific diet or take supplements for a given period of time and a great number of variables are measured before, during and after the intervention period. Most of these studies require external funding, either from governmental or non-governmental research foundations, or private funding. In any case, scientific integrity should be ensured and conflict of interest be avoided. When conflicts of interest could arise, these must be declared and made transparent.

Organizations such as SPIRIT (Standard Protocol Items: Recommendations for Interventional Trials; <http://www.spirit-statement.org/>) propose to describe the role of study sponsors and funders with regard to the design of data collection, data management, data analysis, interpretation of the results, the actual writing of the report, and the decision to submit a report for publication. It should further be indicated whether study sponsor has final authority over any of these activities.

An example of such a statement is “The funding source had no role in the design of this study and will not have any role during its execution, analyses, interpretation of the data, or decision to submit results”.

The “funding source had no role in design etc” is however difficult to maintain fully in practice. The mere fact that the (nutritional) compound in question was studied at all could have been influenced by the sponsor. In most cases, the aim of the research undertaken would have been to only find positive, beneficial effects. In a Cochrane review on the outcome of clinical studies on drug and medical devices, the authors conclude that studies sponsored by manufacturing companies have more favorable efficacy results than studies sponsored by other sources [40,41]. There is no a priori reason to believe this will be different for nutrition studies.

In a recent commentary in the Journal of the American Medical Association (JAMA), Jeffrey Botkin asks the rhetorical question: Should failure to disclose significant financial conflicts of interest be considered research misconduct? [42]. Earlier, in May 2017, JAMA published a thematic issue on conflicts of interest that represented “the multifaceted aspects and complexity of Conflict of Interest from numerous perspectives,” and included 23 papers on this topic. This illustrates how diverse the nature of conflicts of interest is in scientific research. For a research group to have relations with a food (supplement) or nutrition company as such is understandable and even advisable in some cases. The effects of a given (isolated) food component is best studied in the complete food matrix, and for that the producer of the food is indispensable. Also, for communication of the research outcomes to the public at large, such contacts are valuable. It should be clear to all parties involved (researchers, (company) sponsors, regulatory authorities, others) that each one has their own role and responsibility, and that transparency is the rule of thumb. Failure to do so has several consequences. As indicated above, it has been suggested that failure to disclose a financial link of the researcher to the company sponsor should be considered a research misconduct. For a regulatory authority, but in fact for anyone interested in the outcome of scientific research, (financial) links between researchers and sponsors are essential to be reported.

Epilogue

In this Commentary we have touched upon conflicts of interests which may arise in sponsored research into the effects of nutrition on the immune system. In the Introduction, the comparison was made with sponsored art, Jheronimus Bosch's painting *Crucifixion with a Donor* as an example. Out of the 25 paintings in Bosch's name, six have a donor portrayed in them. Out of those six, four are overpainted [43]. It has been suggested that this would reflect the troublesome relation of Bosch with his patrons [44]. Because most of the overpaintings were done later (sometimes many years after the completion of the painting), a more probable explanation is that the painting changed ownership or the work received a new function. For sponsored research these arguments are not valid: whether or not a given sponsor is taken over by another company, whether or not the conclusions of the original paper are challenged by newer research, the conflict of interest will remain.

Conflict of Interest

Author has no conflicts of interest to declare. No (indirect) financial support was applied for or obtained for writing this commentary.

Acknowledgements

Mrs. A. Colley, Mt. Riverview, Australia is acknowledged for allowing to use the photograph in Figure 2. Dr. MF Jonkman, University Medical Center Groningen, The Netherlands provided Figure 3.

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