Towards a new, dynamic concept of HEALTH

Its operationalisation and use in public health and healthcare, and in evaluating health effects of food

Machteld Huber

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Towards a new, dynamic concept of Health

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The content has been elaborated at the Louis Bolk Institute, Driebergen, The Netherlands, an independent international knowledge institute for research and advice to advance sustainable agriculture, nutrition and health.

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Preface

Long before the word *epigenetics* became commonly known, I learned from my agricultural research colleagues how strongly the phenotypes of plants and animals, and their natural resistance, are influenced by various agricultural measures – particularly by the type and amount of plant fertiliser used.

Since then I have become intrigued, from a medical perspective, by the question of whether food products from organisms that maintain an adequate natural resistance might be more beneficial to the health of consumers than products from organisms that are unable to do so and that need external support to survive. In practice, this question translates into the question of whether food products from organic agriculture may have additional health benefits, as this production system is the most commonly used for farming animals and crops with the aim of maintaining the natural resistance of plants and animals, and excluding both pesticides and the preventive use of antibiotics.[1,2]

The health effects of such organic products form a complex question for scientific research, as it touches on several sub-questions and various disciplines. Some of these sub-questions are: What are the factors that influence the relationship between production measures and plant and animal resistance? What factors in plant and animal resistance might affect consumer health? Furthermore, if any influence on human health indeed would occur as a result of the consumption of products with an intact resistance, where and how would this be expressed? What would be the best research design for investigating such effects? What is already known about the health effects from such food products?

To avoid unrealistic expectations and disappointments, please note that this thesis does not deal with the details of all of these questions but only touches on some of the aspects involved.

This topic is very complex; therefore, cooperation with research colleagues has been indispensable. In 2003, a number of Dutch parties took the initiative to establish the International Research Network for Food Quality and Health (FQH).[3] The first FQH initiative was to organise an international, two-day expert workshop with 25 scientists from a broad range of backgrounds, at the Louis Bolk Institute in Driebergen, the Netherlands. These experts discussed the best possible research design for investigating whether organically produced food products would have additional health benefits. It was clear that such research should be broad in design and explorative. There also was consensus on the expectation that íf there was any influence, it would possibly be that on the immune system of young, developing organisms. The argument being that immature immune systems are stimulated by the gut system and their first contact with the external world, namely through food.

Therefore, the choice was made for an explorative, fully controlled, two-generation intervention study, in search of biomarkers, with a suitable animal model (that could be used as a model for humans) for immunological research, applying broad immunological assessments, combined with general health observations, metabolomics, genomics, pathological anatomy and extensive feed analyses.
At the Dutch Wageningen University and Research Centre (WUR), an immunological model for chickens was available, which was chosen for the research design. Immunological lab work was to be performed by the WUR; metabolomics, genomics and food analyses by TNO, and toxicology by RIKILT – most of these parties also being a member of FQH. The Louis Bolk Institute had the lead and was responsible for the production of representative feeds, either from conventional or organic production systems, all blinded.
This study, so far the largest study on this topic, ran from 2006 to 2008, and was financed by the Dutch Government (which was interested the ultimate answer) and two commercial banks. The results were promising; the animals were all 'healthy'– which could be expected, as the nutritional value of both types of feed was adequate – yet many significant physiological differences were found between the feed groups; especially in response to an immunological challenge. Nevertheless, in the end, no conclusions could be drawn from the study, as there was no operational definition of health available by which the phenomena could be interpreted to determine the 'healthier' group. The WHO definition of health, which reads: 'Health is a state of complete physical, mental and social wellbeing and not merely the absence of disease or infirmity', was inadequate for this situation, and scientific literature on the phenomena observed in the research animals was lacking. Although the researchers themselves had a clear idea about which group of animals was healthier, this could not be substantiated scientifically. In the 2010 overview article on this study (see Chapter 9 of this thesis), the concept of 'resilience' was proposed as an interpretation of the observations.
This line of research was not continued 'as it was not possible to draw conclusions'.

Over the same period, there were also other signs pointing to the WHO's insufficient definition of health, for present times, as well as to the need for a more adequate integral concept of health.

- In 2008, biologist Bart Penders of Maastricht University (the Netherlands) published his thesis, entitled 'From seeking health to finding healths'.[4] The thesis posed the question of how large-scale nutrition science works, taken from large-scale cooperative projects that study complex problems. To find an answer to this question, Penders followed two big research programmes on nutrition and health; namely the Dutch Gut Health programme and research by the EC funded European Nutrigenomics Organisation (NuGO). He described how researchers, in order to make operational modules to solve a complex problem, had modified certain elements of that problem, suitable for their research facilities. This also concerned the notion of health, which finally resulted in a diversification of notions, as well as in co-existing standards for health, according to the pluriformity of institutional settings. Penders concluded that the synthesis of these diverse notions and health standards was difficult, and that this difficulty should be realised by policymakers, who often expect more from research than would be realistic.

- In 2008, Alex Jadad from Canada called for a global conversation in the BMJ[5] and hoped, through his blog, to revitalise the discussion of 60 years ago that led to the WHO definition, but this time through the power of social media. Jadad and his colleagues conducted a literature study and concluded that the founding ideals of the WHO – to eradicate diseases and lead humanity towards overall well-being – remained unfulfilled and were unlikely to be achieved in the following decades. Although an increase in longevity as well as a decrease in infectious diseases have been realised, there has been an overall increase in lifestyle-connected chronic diseases, especially in low- to middle-income countries. According to Jadad, criticism on the WHO definition was increasing in the literature, and he wondered if it would be possible at all to reach a basic level of agreement on the meaning of the word health. Is health a construct that can be defined and measured? Can any definition of health be operationalised? Doubtful of this possibility himself, he nevertheless invited BMJ readers to either comment on and challenge the WHO definition or try to enhance it.
- In March 2009, an editorial appeared in the Lancet, entitled 'What is health? The ability to adapt'[6], introducing a series on health in the occupied Palestinian territories. The editor criticised the WHO definition as being too utopian, within the context of modern understanding of disease and the diversity of risks, and stated that risk-free well-being is impossible. He suggested the already mentioned physical, mental and social domains should be extended by two: planetary biodiversity and the earth's climatic state. Human well-being and health are highly contingent on the condition of both domains. He cited the French philosopher-physician George Canguilhem, who in 1943 described health not as a fixed entity, to be defined statistically or mechanistically, but as the ability to adapt to one's environment. Health is defined, not by the doctor, but by the individual according to his/her functional needs. The role of the doctor, according to Canguilhem, is to help the individual adapt to their unique prevailing conditions.[7]

I decided to shift my focus towards the concept of health as a result of this accumulation of indications that the prevailing definition of the WHO, however idealistic, is not sufficient to support scientific development, nor for the medical profession. In addition, there is the current lack of an integral concept of health by which a diversity of laboratory outcomes in studies on health effects from nutrition can be interpreted. Health already was a topic that has had my special interest since my own encounters with disease. As John Lennon said: ‘Life is what happens, when you’re planning other things’. This thesis is an illustration of that statement. The composition of the text aims for an outline based on logic and is therefore not fully chronological.

Machteld Huber

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Chapter 1

General introduction and outline of this thesis

Introduction

Health has always been an important value for human beings.[1,2,3] But what is health? How is it defined?

The English word 'health', etymologically, means wholeness, being whole, complete, sound, well. To 'heal' literally means to make whole. Both words go back to the ancient Germanic word 'hailiz', which is the source for the Old English word 'hæl' and the Old High German word 'heil', which all mean the same.

Although the language was different, the perception about health and well-being as a form of wholeness was quite similar in ancient Greece. The physician Hippocrates (approx. 460–370 BC), today still considered the 'father of Western medicine', described 'good health' or 'physis' *(φύσις)* to be the natural situation: a state of balance between different 'elements'. Aristotle (384–322 BC), the 'father of Western philosophy', described 'eudaimonia' (εὐδαιμονία) to be the final goal and good for man. Eudaimonia in modern translations is described as 'personal well-being' or 'happiness'. It is not a static state but a continuous process of development and personal growth, of fulfilling one's potential and to flourish.

These views of Hippocrates (and his Greek-Roman follower Galenus) and Aristotle about the human potential to be in a state of balance and the virtue of developing oneself remained highly influential in Western medicine and culture during 15 centuries AD.

A different way of interpreting human health and disease emerged in medical thinking around the 16th century, when studies on the anatomy of the human body started to appear. This new analytical approach continued to be developed until the second half of the 19th century, when it fully superseded the qualitative way of thinking and condensed into the paradigm of cell physiology, microbiology and pathological anatomy as the dominant view in medicine. Health became the 'absence of disease'. However, in spite of this new paradigm in medicine, philosophers of that time maintained a broader view of health than merely the absence of disease. For example, Goethe (1749–1832) and Nietzsche (1844–1900) described the virtue of ongoing personal development, for which illness can even be a stepping stone, towards the full acceptance of life, which Nietzsche called the 'große Gesundheit' ('great health').

The presently still prevailing definition of health is that of the WHO, from 1948, which states: 'Health is a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity'. At the time of its formulation, it was ground-breaking because of its broadness, mentioning more than just physical health and more than just the absence of disease. It appeared to combine the qualities of balance and wholeness that physicians and philosophers had addressed in earlier times. The authors of the WHO Constitution intended this definition to be an ideal to strive for. Yet, from the start, there has been criticism over this formulation, which since has only increased. Most of the criticism concerns the word ‘complete’, because when exactly has a person reached completeness in all these domains? The required ‘completeness’ would mean that, in fact, hardly anyone is ever healthy, which brings as a consequence the need or justification for treatment without end. In other words, the definition, unintendedly, shifts the focus back to health as the ‘absence of disease’ and, thus, it medicalises. From the perspective of the present dominance of chronic diseases, as opposed to the 1948 situation when infectious diseases were the prevailing problem, this implies long-term treatment – aimed at reaching the state of complete well-being.

Another point of criticism concerns the word ‘state’. Does life not present people with new challenges on a continuous level, and is the ability to adapt, such as on a physical level also expressed by the immune system, not a basic feature of the healthy human being?

If so, this implies that the human reality, be it physical, mental or social, is dynamic rather than static.

Yet another problematic feature of the definition is that it is difficult to operationalise. What standards and cut-off points should one choose to measure a state of ‘complete’ well-being?

So, notwithstanding the utmost respect for the ideals with which the WHO definition was once conceived, an update of the formulation needed to be considered.

The main problems to overcome are the stimulus towards medicalisation, the static character of the definition and the problems related to its operationalisation.

An issue that is directly related to the definition of health is the question of how to identify health effects that are related to nutrition. Nutrition was already promoted by Hippocrates as an important determinant of health, and today this potential of nutrition also is fully recognised. However, when studying such health effects, the WHO definition is not applicable. Modern nutritional research generally uses two approaches. The first of which is to analyse the compounds of a particular food product, and compare the results to the state of knowledge about the recommended daily intake. This results in a statement about the ‘nutritional value’ of this food product. Despite the traditional success in defining and understanding nutritional deficiencies, this approach has its weaknesses, as the effects of the consumption of nutrients are not always predictable. Will the compounds be absorbed, will they become biochemically active, or are they excreted without effect? The study of kinetics and dynamics in nutritional physiology is supportive here, to a certain extent. However, in order to deal with this uncertainty, another, second type of research also is performed, in the form of various types of consumption studies. In such studies, the effects from consumption are evaluated in humans and/or animals, or using laboratory models. This results in statements about the food's 'health value', but these outcome parameters are not connected to the WHO definition of health as 'complete well-being'. In most cases, the studies focus on possible, preventive effects from nutrition on diseases, or on early signs of diseases. This means that, in fact, also in the field of nutritional research, health is still defined as the 'absence of disease', on the physical level.

This is indeed a problem, and for the following reason. If, as described before, the human reality is that of continuous adaptation to external challenges, the relevant question is whether nutrition could support this adaptability and thus enhance resilience. To study such an effect from nutrition, research is needed at an earlier stage, before the first signs of disease appear. This means researching healthy organisms and the possible enhancement of health. Such research is possible, but needs a more dynamic approach than the traditional, namely by challenging an organism, studying the subsequent reaction and the so-called 'phenotypic flexibility'; for example, in the immune system. This approach allows studying the effects from nutrition on adaptability and resilience, and is also useful to answer the question of whether organic foods are healthier than conventional products; a topic of high societal interest, but also of much scientific controversy. Producers who use organic production methods aim to enhance the natural resistance of plants and animals and, thus, to increase their adaptability and resilience. In organic agriculture there is the perceived sequential relationship of healthy soil leading to healthy plants which leads to healthy animals and finally to healthy consumers[5]; in other words, the expectation that resilience enhances resilience. To overcome the emotions connected with this topic, sound research is necessary. Such resilience research could be very relevant, not just on the topic of organic nutrition, but also on health effects from nutrition in general, as it provides ways to study the effects from health promotion. And, coming back to the notion of health, for this type of research a dynamic definition or concept of health is very supportive. Not as utopian as the WHO definition, but still describing health as being more than the 'absence of disease'.

This thesis describes the issues around finding a more appropriate definition of health for present times and its operationalisation in healthcare and public health, as well as for nutritional research. This last topic is further elaborated with regard to the question about the possible health effects of organically produced food.

The issues of health and nutrition are addressed according to the following research questions:

1. *Health: How has this basic value for mankind been defined in history, by physicians as well as philosophers, until recent times? What were the intentions of the WHO, when defining health in 1948? Could the WHO intentions be maintained, albeit in a new, more dynamic conceptualisation of health? How is this newly proposed*

concept of health evaluated by the various stakeholders in healthcare, and how could it be further operationalised for the future?

2. *Nutrition: It is a precondition for human life and functioning and, from various perspectives, poses challenges for mankind. How has nutrition been perceived and studied in history, until recent times? What does the newly proposed dynamic concept of health mean for the evaluation of health effects of foods in general and, more specifically, of organically grown foods that are produced according to a systems approach?*

The thesis elaborates these research questions accordingly; divided into two sections:

PART I - HEALTH
and
PART II - NUTRITION and HEALTH

The outline of this thesis

The **Preface** describes the background for this unusual combination of topics.
Chapter 1 gives a general introduction and outline of this thesis.

PART I - HEALTH

Chapter 2 provides a concise overview of the literature on how 'health' has been understood by physicians, as well as by philosophers, in history up to present times, and describes the establishment of the WHO, its ideals and connected definition of health. In the distant past, health was perceived as a balance of qualities and 'wholeness'. Then, the paradigm of cellular, molecular and sub-molecular pathogenesis developed and health became the 'absence of disease', whereas recently, a more integral perception of health again has been gaining recognition.

Chapter 3 describes the outcome of a two-day international conference, held in the Netherlands, with the objective of achieving a new formulation of health. The main critics of the WHO definition of health, as well as elements of this definition which should be maintained in the newly introduced dynamic concept of health, are described.[6]
Chapter 4 presents research that elaborates on the new concept of health, as introduced to a diverse population of stakeholders in the field of healthcare and public health, in order to establish first steps towards a conceptual framework.

PART II - NUTRITION and HEALTH

Chapter 5 contains an introductory description of the developing views on nutrition and health, as well as present-day challenges related to nutrition. An overview of the prevailing methods and views in evaluating the health effects from food is provided and limitations are discussed.

Chapter 6 sketches different research approaches for studies on the health effects from nutrition, and reviews the limited number of research results available on possible health effects from organically produced food, compared to those of conventionally produced food.[7]

Chapter 7 zooms in on the question about possible health effects from organic and conventionally produced products, reviews feeding trials with animals, more in detail, and discusses the connected research dilemmas.[8]

Chapter 8 elaborates on the new concept of health, as introduced in Chapter 3, in a first step to establish methods to evaluate health effects from foods in general, which could also be used for organic foods. Furthermore, it describes challenges that could be introduced, in order to study the adaptability of organisms.[9]

Chapter 9 presents the exploratory, two-generation intervention study in chickens, as a model for humans, in search of biomarkers for health effects from organically produced food, compared to conventionally produced food. In this study, immune challenging was used to compare the health of the animals.[10]

Chapter 10 provides a detailed description of one of the analyses on the animals discussed in Chapter 9: the genomics of the jejunal gut.[11]

Chapter 11, the general discussion, reflects on the underlying theme of this thesis and summarises the main findings of the earlier chapters, in connection to the research questions. Methodological strengths and weaknesses of the performed studies are subsequently considered, followed by reflections on their results, including a framework of themes to be further elaborated, and a conclusion.

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PART I

HEALTH

Chapter 2

The concept of health through the ages and the WHO definition of health

(A slightly adapted version of this chapter will appear in Elsevier's International Encyclopedia of Social & Behavioral Sciences, 2nd Edition)

Introduction

Throughout history, health has been an important value for human beings. But what is health? How is it defined? This chapter provides a concise overview of the literature on how 'health' has been understood by physicians, as well as by philosophers, in history up to present times, and describes the establishment of the WHO, its ideals and connected definition of health.

Etymology

The English word 'health', etymologically, means wholeness, being whole, complete, sound, well. To 'heal' literally means to make whole. Both words go back to the ancient Germanic word 'hailiz', which is the source for the Old English word 'hæl' and the Old High German word 'heil', which all mean the same. The ancient Greek word for health was 'euexia', which means being in good condition.

Historical definitions of health

Ever since human documentation began, expressions can be found where differentiations are made between the human state of well-being and 'illnesses', the latter considered to be abnormal, undesirable and to be healed, if possible. In different ancient philosophies (e.g. Chinese, Indian, Egyptian or Greek) concepts of illness and of the state of 'good health' are described, as well as the ways in which human good health could be achieved.[1] According to these concepts, good health is conceptualised as a state of balance between different 'elements', which represent different qualities, e.g. the five elements: wood, fire, earth, metal and water and the Yin and Yang qualities in Chinese philosophy (Taoism); or the four humours in Greek medicine (black bile, yellow bile, phlegm and blood) that originated from knowledge from ancient Egypt and Mesopotamia.

Health is described as achievable and influenced by lifestyle habits and, under certain circumstances and if fate allows, as influenced by god(s) and mediated by priests in temples. 'Hygeia', the goddess and personification of health, cleanliness and hygiene in ancient Greece, represented a healthy way of living. She was one of the six daughters of 'Asklepios', the god of medicine and healing.

Physicians and philosophers

Over the course of history, physicians as well as philosophers developed views on the nature of health. Hippocrates (approx. 460–370 BC) is still considered 'the father of Western medicine', as he was the first to emphasise the importance of a good physical examination of the patient; he created a new paradigm, discriminating four humours in humans: black bile (*melan chole*), yellow bile (*chole*), phlegm (*phlegma*) and blood (*haima*), each corresponding to one of the traditional four elements and temperaments.[2] A healthy situation was the balance between the four humours, and Hippocrates considered diet the most influential lifestyle factor according to which the relative proportions between the four humours could be restored, in addition to the influence of climate, wind, and the quality of water and soil.

The historically even more influential Greek-Roman physician Galenus (AD 131–216) followed and refined the teachings of Hippocrates and described six lifestyle factors (‘*res non naturalia’)* that should be in balance in order to maintain health: food and drink (*cibus et potus*), being asleep and awake (*somnus et vigilia*), light and air (*aer*), secretions and excretions (*secreta et excreta*), work and relaxation (*motus et quies*), and emotions (*affectus animi*).

The ‘father of Western philosophy’, Aristotle (384–322 BC), does not discuss ‘health’ as such, but states that extremes in the bodily condition should be avoided and maintaining a proper balance (the mean) is a virtue.[3] Yet, in his ethics, he considers ‘*eudaimonia*’ (well-being) the final goal and ‘final good for man’. Eudaimonia is an important concept, as it is rediscovered in modern views on health. Eudaimonia literally means ‘the state of having a good indwelling spirit, a good genius’; in modern translations it is described as happiness, personal well-being. Eudaimonia is not a static state but a continuous process of development to fulfil one’s potential and to flourish. This implies self-realisation, through which humans may achieve this happiness and personal well-being.

The views of Hippocrates and Galenus, as physicians, and Aristotle, as a philosopher, about the human potential to be in a state of balance and the aim of developing oneself, remained highly influential in Western medicine and thinking, over 15 centuries.

From the 16th century - a new paradigm dawns in medicine

The physician’s view

A different way of interpreting human health and disease started emerging in medicine when Vesalius (1543) published his studies on the anatomy of the human body.[4] These studies were based on his own observations during the dissection of bodies, which was considered sacrilege, until then. A century later William Harvey (1628) described the system of blood circulation, which until then was thought to be a tidal movement, similar to the flood and ebb tides.[5] The Galenic humouralism was definitively displaced in 1858, when Rudolph Virchow published his theory on cellular pathology.[6,7] In the same period, Semmelweiss discovered the protective effect of disinfective measures against deathly puerperal fever; soon thereafter Pasteur described the existence of bacteria, which he could see in the microscope, and ways to disarm them (e.g. by pasteurisation). From then on, the paradigms of cell physiology, microbiology and pathological anatomy became the dominant views in medicine. Diseases were no longer understood as caused by misbalances of qualities, but were to be searched and understood in the physical body, in organs, cells and microbes, and should be treated there. Health definitively became the absence of disease.[8]

Parallel to this transition in medical thinking, public health developed in the fast growing cities, due to the industrial revolution, and was expressed in the form of better nutrition, provision of clean drinking water, sanitary measures, waste disposal, as well as the first vaccination programmes. Infectious diseases, such as cholera, typhus and small pox, were eradicated and became extinct. The new approach proved to be very effective, although local authorities required a fair amount of convincing by medical doctors about the need for public health measures. In the Netherlands, it took several laws implemented by the central governments (e.g. by the Thorbecke Government) of 1865, 1872 and 1901, as well as a strong lobby from physicians (called hygienists and later radical hygienists), before public health became seriously integrated in local communities.[9]

Contemporary philosophers of the 18th and 19th century

In the period of this paradigm shift in medical thinking, philosophers (and poets) still expressed a broader perspective on health than seeing it merely as the absence of disease. The following quote is by Goethe (1749–1832): 'What is the highest virtue on earth? To be healthy? No: to become healthy'.[10]

Nietzsche (1844–1900) discriminated between 'small health' and 'big health'.[11] While 'small health' according to his view was concerned with the daily derangements and illnesses that doctors dealt with, attaining 'big health' meant saying a wholehearted YES to life, with all its insecurities, tragedies and finally death. Big health includes small health, but is connected to an inner growth that goes far beyond that.

Rilke (1875–1926) had a weak personal health, but regarded his illnesses in a positive way, namely as something that was supporting his personal growth. He stated 'Illness is the means by which my organism frees itself from unfamiliarity. I need just to support my body in being ill, because that is my development'.[12]

Health in the 20th century, the foundation of the World Health Organization and its definition

From the 20th century onwards, public health started to be organised on both a national and international scale, operated by large health organisations.[13] In 1902, the international organisation Pan American Sanitary Bureau (PASB) was founded in Washington DC, and in 1907 the Office International d'Hygiène Publique (OIHP) followed, with headquarters in Paris. In 1913, the Rockefeller Foundation, the first non-governmental organisation (NGO) in this field, was initiated with the aim to enhance the well-being of all humankind.

After World War One, the League of Nations Health Organisation (LNHO) was established, which stood out as the first official body with a global perspective and the broadest work spectrum. In 1919, the League of Red Cross Societies was organised, which developed health programmes with an international outlook.

In 1943, during World War II, a Health Division of the United Nations Relief and Rehabilitation Administration (UNRRA) was initiated by US President Roosevelt 'for the relief of victims of war in any area under the control of any of the United Nations through the provision of food, fuel, clothing, shelter and other basic necessities, medical and other essential services'. The term 'United Nations' referred to the Allies of World War II.

The Second World War led to the wish to establish a world organisation such as the present-day United Nations, to preserve world peace. When in 1945, in San Francisco, representatives of 50 nations met to draft the Charter of the future United Nations, there were three medical doctors among them, from Norway, Brazil and China. They met one day for a 'medical lunch', where the Norwegian physician, Karl Evang (described as the most active of the three), suggested to start a new health organisation.[14] The idea was to unite the different existing organisations into one single health organisation. The three succeeded in having the word 'health' inserted in the Charter of the United Nations and they recommended the general conference to establish an international health organisation. The recommendation was approved unanimously, and this was the beginning in 1945 of the World Health Organization.

A Technical Preparatory Committee was established to prepare a draft constitution for this WHO. This Committee of 16 men plus advisors met in Paris on 22 separate occasions, between March and June of 1946.[15] Their work resulted in the draft Constitution of the WHO, which was signed on 22 July 1946 by the representatives of 61 countries at the International Health Conference in New York. Thereafter, all countries were asked to ratify the Constitution, which was completed in 1948.

Between 1946 and 1948, an interim committee was set up to ensure continuity, presided over by Andrija Stampar of former Yugoslavia. This committee decided in favour of Geneva as the seat of the headquarters of the WHO, and it was in Geneva, on 7 April 1948 at the first World Health Assembly that the WHO Constitution came into force.

The Constitution of the WHO

The Constitution of the World Health Organization starts with the Preamble, stating the principles upon which the whole document is based, and begins as follows:

> *'The States parties to this Constitution declare, in conformity with the Charter of the United Nations, that the following principles are basic to the happiness, harmonious relations and security of all peoples:*

> ***Health is a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity.***
> *The enjoyment of the highest attainable standard of health is one of the fundamental rights of every human being without distinction of race, religion, political belief, economic or social condition.*
> *The health of all peoples is fundamental to the attainment of peace and security and is dependent upon the fullest co-operation of individuals and States'.*[16]

The definition of health has a central position in the Preamble to the Constitution of the WHO.
There had been some discussion about whether a definition of health should be in the Constitution.
Szeming Sze, a public health doctor from China, one of the three who initiated the idea for the WHO and a member of the Technical Preparatory Committee, describes how the phrasing of the definition of health came about.[14] Some detail on this process can also be found in the minutes of the Technical Preparatory Committee.[15] A sub-committee of four physicians: Canadian psychiatrist Brock Chisholm (later to become the first Director-General), Argentinian psychiatrist Gregorio Bermann, Professor of Hygiene Joseph Cancik from Czechoslovakia, and Sze himself had 'some pleasant academic discussions' and decided to emphasise mental health as well as the preventive side of healthcare. Thus, they came to the wording of health as more than merely the absence of disease. The minutes of the Technical Preparatory Committee include several draft versions of the definition, including terms such as 'physical fitness' and 'positive health'.

Idealistic intentions

The WHO definition of health is phrased very idealistically, as is the Constitution as a whole. It is important to realise that these formulations were a reaction to the horrors of World War II. The atmosphere in the Preparatory Committee may be characterised by a phrase in the Committee's minutes, describing a speech by Dr Chisholm:

> *'The world was sick, and the ills from which it was suffering were mainly due to the perversion of man, his inability to live at peace with himself. It was in man himself that the cause of present evils should be sought. The microbe was no longer the main enemy: science was sufficiently advanced to be able to cope with it admirably, if it were not for such barriers as superstition, ignorance, religious intolerance, misery and poverty. These psychological evils must be understood in order that a remedy might be prescribed, and the scope of the task before the Committee, therefore, knew no bounds. For that reason, Dr Chisholm associated himself with the "visionaries". What was taking place in these meetings would be*

> *of great historical importance if all members aimed at universal and worldwide achievement. To do this, they might find it necessary to bite off more than they could chew, but the alternative was complete chaos. They should do their utmost to bring all the peoples of the world together in the service of physical, social and emotional health'.*[17]

When Chisholm stated that 'the microbe was no longer the enemy', he referred to penicillin, which recently became available for general use, after Fleming's discovery in 1928. In a time where infectious diseases were still dominant, this discovery raised great expectations of banning them. Yet, the broadness of the definition of health, beyond being the absence of disease, was ground breaking and owed to this special group of physicians, who directed this process of establishment.

In several charters, declarations and statements, the idealistic aims of the WHO were further elaborated every few years since the inception of the definition of health.[18] The best known of these is the 'Alma Ata Declaration', which emerged from the UNICEF/ WHO Conference on Primary Health Care of 1978, addressing health as a fundamental human right, health inequalities and the connected need for economic and social development; the 'Health for All by the year 2000' declaration of 1981, as a target for the WHO; and especially the condensed and strongly formulated 'Ottawa Charter for Health Promotion' of 1986. There, the WHO definition was described in connection to the conditions by which this state could be met: 'To reach a state of complete physical mental and social well-being, an individual or group must be able to identify and to realise aspirations, to satisfy needs, and to change or cope with the environment. Health is, therefore, seen as a resource for everyday life, not the objective of living. Health is a positive concept emphasizing social and personal resources, as well as physical capacities. Therefore, health promotion is not just the responsibility of the health sector, but goes beyond healthy lifestyles to well-being'. As fundamental prerequisites for Health are mentioned: peace, shelter, education, food, income, a stable ecosystem, sustainable resources, social justice and equity. The charter calls for 'building healthy public policy' and the 'creation of supportive environments'.
During the 2013 Helsinki 8th Global Conference on Health Promotion, the tone was somewhat less idealistic and the 'implementation gap' was addressed. The 'Health in All Policies' approach was concluded to be one of the key domains of shared governance towards health in the 21st century. In all these texts the original definition of health of the WHO was maintained.

Operationalisation of health by the WHO since 1948

The WHO Classification systems

The WHO monitors population health around the world and, thus, needs health indicators. Yet, the WHO itself recognises that the definition is not sufficient to develop operational indicators of health. Chatterji *et al.* describe the conceptual basis on which the WHO searches for ways to measure and report on health, and mentions three intercultural consensus points about health, as the basis for face validity[19]: 1) health is a separate concept from well-being, of intrinsic value to human beings, as well as instrumental in well-being; 2) health comprises states or conditions of body and mind and therefore attempts to measure need to include measures of body and mind function; and 3) health is an attribute of an individual person though aggregate measures of health may be used to describe populations or aggregates of individuals.'

There is general acceptance about describing health states of individuals in terms of multiple domains and in self-report instruments. Valid, reliable and comparable indicators are needed, for the purpose of health status measurements. According to the WHO, a measurement instrument requires:

- classification of health status domains;
- specification of a set of domains necessary and sufficient to describe health states for measurement purposes;
- specification of what is being measured in each domain;
- common understanding of what constitutes full health, vs. an exceptional talent in any given domain;
- and, if required, construct summary measures of the average level of population health, a method to place a single cardinal value on the overall level of health associated with a health state, defined in multiple domains.[19]

Since 1948, the WHO has designed several classification systems, together described as the WHO Family of International Classifications (WHO-FIC).[20] At first, the focus was on causes of mortality, diagnoses and morbidity, for which the International Classification of Diseases and Related Health Problems (ICD) was developed, which is the international standard for epidemiological purposes.

For use in primary care, the International Classification of Primary Care (ICPC) was developed, in which components from three former classifications were combined on the topics: reason for encounter, process in primary care and health problems in primary care (ICHPPC-2-d).[21,22,23]

Gradually, the thinking about health states evolved, and in order to not only evaluate mortality and diseases, but also disabilities, functioning and health states, the International Classification of Functioning, Disability and Health or ICF (previously the ICIDH) was elaborated (Figure 1). The ICF measures six domains: 1) existing disorders or diseases; 2) body functioning, related to different organ systems; 3) activities in daily life; 4) societal participation; 5) environmental factors in the physical and social environment; and 6) personal factors. A person's capacity and performance is measured and the gap between capacity and performance is considered to reflect the impact of the actual environment. The domain of personal factors (marked yellow in Figure 1) – the least elaborated – is under discussion, because there are so many social and cultural differences influencing this domain. Arguments are formulated in favour of addressing aspects such as 'personal empowerment' or 'meaningfulness' in this domain, but meet with objection from people who fear situations where a person might then be blamed for health damage, or for not being able to cope, whereas in fact a difficult external situation is the main cause rather than a lack of meaningfulness. This may result in people being treated unfairly or being blamed, while in reality they are victims of circumstance.

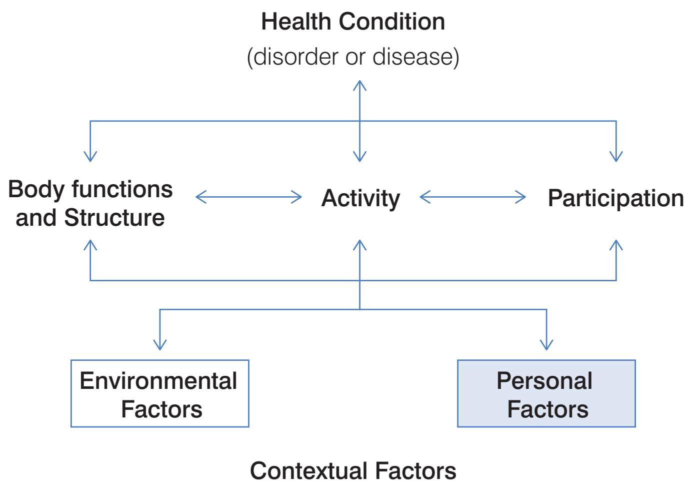

Figure 1 The different domains included in the International Classification of Functioning, Disability and Health (ICF)

The WHO's measurements of Quality of Life and Well-being and Health-Related Quality of Life

Objective indicators of health and functioning as specified in the ICF need to be distinguished from a person's subjective appraisal of well-being and quality of life. A person with certain impairments may very well still experience a good quality of life; this is called the 'disability paradox'.[24] In this context, the WHO identifies 'Quality of Life' (QOL) as 'an individual's perception of his position in life in the context of the culture and value systems in which they live, and in relation to their goals, expectations, standards, and concerns'.[19]

The WHO has developed several instruments for measuring well-being and quality of life: the WHOQoL-100[25] with 100 items covering six domains and the WHOQoL-BREF[26] with 26 questions in four domains, which worldwide are among the most widely used instruments for assessing Quality of Life. The short WHO-5 well-being index[27] connects well-being mainly to mental health.

General Quality of Life should be distinguished from Health-Related Quality of Life (HRQoL), i.e. quality of life perceived in relation to symptoms, impairments and functions. For HRQoL, the best known measurement instruments (from outside the WHO) are the SF-36 and the Nottingham Health Profile (NHP) (both earlier than the WHOQoL), the Health Utility Index (HUI) and the EQ-5D (both later than the WHOQoL).

To support policymakers in the field of human health in their decisions about expenditures, measurement and calculation approaches have been developed, such as the Quality Adjusted Life Year (QALY) and the Disability Adjusted Life Year (DALY), with the aim to come to a single, common metric for assessing cost utility. The QALY is developed as a measure of health gain, used for cost-utility and cost-effectiveness analysis, to estimate the efficiency of preventive and curative healthcare services.[28] A QALY is a unit, including the quality and quantity of the life lived. The number of QALYs lived over a certain period is the number of years lived in that period, multiplied by a correction factor for quality of life. A factor of 1.0 means no burden at all, and death is considered equivalent to 0.0 (although some health states can be considered worse than death and may have scores below zero). Cost-utility analysis looks at the amount of QALYs that count, not at the number of (uncorrected) years. The DALY is a measure for the loss of health and the overall burden of disease or disability. It is, in fact, a 'reversed QALY'.[29] In literature the QALY is generally considered to be the best developed and theoretically best underpinned outcome measure for cost utility. Yet, there is (since 35 years and still ongoing) a lot of debate connected to the QALY, as well as to the DALY, although also the critics state that for QALY no better alternative is available yet. Critics connected to both QALY and DALY are divers but similar, like about the choice of group who values health gain, estimates the quality of life, or the burden of a disease. Can this be the general public, or even healthcare providers, or should it be patients? How about effects of cultural background, social level, education and age, as well as differences between illnesses and individuals? A crucial issue is that of how to reliably measure and quantify quality of life and disability, to which there are different approaches and opinions. In fact, many critics state that it is impossible to reach one universal 'objective' measure, with which policy can work; it remains a matter of values and stakes.[30]

Contemporary philosophers of the 20th and 21st century on human health

The WHO was designed and further developed mainly by members of the medical profession and public health, and health policymakers. Yet, 'health' is not a topic that concerns only medical professionals – it concerns humankind as a whole, and since the establishment of the WHO also philosophers have thought about health. Some influential philosophical views should be mentioned.

In 1975, Christopher Boorse presented his Bio-Statistical Theory (BST)[31,32], theorising that 'health' is a state within the range of what constitutes typically normal functioning and thresholds within the organism; consequently, disease is abnormal functioning. Health, thus, is the absence of disease, with a strict demarcation. Normal functions can be discovered empirically and statistically by the natural sciences and are thus objective and value-free, according to Boorse. We do need to develop enough and appropriate reference classes, connected to these functions. Despite widespread criticism, the appeal to develop reference classes has since been commonly accepted in healthcare, and Boorse's theory has reached the status of background theory of health and medicine.[33]

Out of discontentment with the prevailing view of health being the absence of disease, the Swedish philosopher Lennart Nordenfelt in 1987 published his theory of health as 'the ability to achieve vital goals' (revised in 1995).[34]

Nordenfelt described a person's vital goal as 'a state of affairs that is such that it is a necessary condition for the person's minimal happiness in the long run.' The stipulation of 'in the long run' is aimed at avoiding the definition of health being centred on immediate pleasure, but, instead, being more in line with long-term happiness, thriving, or flourishing, such as Aristotle's *eudaimonia*. More recently (1995), Nordenfelt rephrased his definition of vital goals as 'a state of affairs which is either a component of or otherwise necessary for the person's living a minimally decent life. This includes more than survival'.[35,33]

In his paper of 2013, Venkatapuram proposes a modification of Nordenfelt's conception of health.[33] He criticises Nordenfelt's definition in that it conceptualises vital goals as social, cultural and ethically relative and thus subjective and not normative. In addition, Venkatapuram indicates that Nordenfelt does not specify a set of vital goals. Venkatapuram, however, describes a set of basic vital human goals, or 'central human capabilities and functioning', inspired by the capabilities approach of Martha Nussbaum that set a norm.

Venkatapuram reasons that 'health is a person's ability to achieve or exercise a cluster of basic human activities'. He refers to Nussbaum's 10 basic capabilities needed to live a life worthy of human dignity: 1) being able to live a normal lifespan; 2) having good health; 3) maintain bodily integrity; 4) being able to use senses, imagination, and think; 5) having emotions and emotional attachments; 6) possessing practical reason to form a conception of the good; 7) having social affiliations that are meaningful and respectful; 8) expressing concern for other species; 9) being able to play; and 10) having control over one's material and political environment.

It may be noted that Venkatapuram introduces circular reasoning under 2) by stating that 'being in good health' is one of the capabilities that is conditional for his definition of health. Venkatapuram stresses that these 10 capabilities, as moral entitlements emanating from a person's human dignity, should become the source of political principles for a liberal pluralistic society. He states that 'ensuring that each member achieves a threshold level of these ten central capabilities, should become a primary political goal'. He expects different societies to define threshold levels for each capability, depending on their history and resources, and thus defining health as a minimal conception of human well-being.[33] Here, Venkatapuram apparently contradicts himself, by criticising Nordenfelt for his contextually relative goals, while stating that the thresholds for his 'norms' should be set, relating to the different societies. Yet, a clear ethical statement is being made, which is similar in intention to the ideals of the WHO. It is remarkable that the content of a philosophy, such as the one by Boorse about objective reference classes, reached the medical establishment, whereas the philosophies by Nordenfelt and Venkatapuram, to date, have not crossed – in dissemination of their thoughts – the apparent gap between philosophy and medicine.

More health concepts outside the WHO in the 20th and 21st century

In the 20th and 21st century, also non-philosophical professionals outside the WHO thought about the definition of health, and the role of the physician in this perspective, some of whom are mentioned below.

In 1941, Henry Sigerist, a Swiss-born American medical historian, analysing the relevance of health for human welfare, stated that 'A healthy individual is a man who is well balanced bodily and mentally, and well-adjusted to his physical and social environment. He is in full control of his physical and mental faculties, can adapt to environmental changes, so long as they do not exceed normal limits, and contributes to the welfare of society according to his ability. Health therefore is not simply the absence of disease; it is something positive, a joyful attitude towards life, and a cheerful acceptance of the responsibilities that life puts upon the individual'.[36]

In 1943, French physician Georges Canguilhem stated that 'Health is not defined by the doctor, but by the person, according to his or her functional needs. The role of the doctor is to help the individual adapt to their unique prevailing conditions. This should be the meaning of personalised medicine'.[37]

In 1972, Irving Zola, American activist and writer in the field of medical sociology, gave the critical description that medicine was becoming a major institution of social control, nudging aside, if not incorporating, the more traditional institutions of religion and law. According to Zola, it was becoming the new repository of truth, the place where absolute and often final judgments were made by supposedly moral neutral and objective experts. These judgements were made, not in virtue or legitimacy, but in the name of 'health'. Moreover, this was being accomplished by 'medicalising' much of daily life, by making medicine and the labels of 'healthy' and 'ill' relevant to an ever increasing part of human existence. This, according to Zola, was rooted in the increasingly complex technological and bureaucratic systems, which led people to rely on experts.[38]

In 1975, Ivan Illich, Austrian philosopher, Roman Catholic priest and critic of the institutions of contemporary Western culture, described health as 'the ability to adapt to a changing environment, to growing up and to aging, to healing when damaged, to suffering and to the peaceful expectation of death. Health embraces the future as well, and therefore includes anguish and the inner resources to live with that anguish'.[39]

From 1977 onwards, J. André Knottnerus, GP and epidemiologist, argued that one general definition of health was useless, as it should not be the physician but the patient, with his individual experience of well-being and healthiness, who decides what health is. It is the role of the physician to identify and to fight, together with the patient, the factors that hinder the patient's specific feelings of well-being and health.[40] In 1983, he reconfirmed this view about the patient's perception and his needs, as being decisive where it concerns health, instead of the regular medical model. He strongly argued in favour of an integrated approach by physicians, including an eye for social problems, and not just a reduced biomedical approach. By that he opposed the often heard societal accusations and fears that such an integrated approach would be equivalent to medicalising problems that are not biomedical.[41] In 1986, Knottnerus elaborated the topic of clinical diagnosis, and described that diagnostic reference values could not be generalised but should be related to the situation and the aim of testing.[42] In 1988, he argued that the principally integral and personalised approach, in general practice, needed specific methodological requirements to be met in order to develop scientific evidence for interventions.[43]

Stokes, Noren and Shindell, in 1982, described health as 'a state characterised by anatomical, physiological, and psychological integrity; an ability to perform personally valued family, work, and community roles; an ability to deal with physical, biological, and psychological stress; a feeling of well-being; and freedom from the risk of disease and untimely death'.[44]

In 1986, René Dubos, a French-born American microbiologist and humanist (best known from 'Think global, act local') stated that, in his view, 'Health is a modus vivendi enabling imperfect men to achieve a rewarding and not too painful existence while they cope with an imperfect world… Health and vigour can be achieved in the absence of modern sanitation and without the help of Western medicine. Man has in his nature the potentiality to reach a high level of physical and mental well-being without nutritional abundance or physical comfort'.[45]

In 1995, John M. Last, Australian-born Professor of Public Health, described health as ‘a state of equilibrium between humans and the physical, biological, and social environment, compatible with full functional activity’(2nd edition 1998).[46]

In 1996, Alvin R. Tarlov, UK-born Professor of Medicine in the US, analysed the definitions of health of the past half century and concluded there to be a remarkable consistency of the following three conceptual components:

1. The capacity to perform (relative to potential);
2. The achievement of individual fulfilment; the pursuit of values, tasks, needs, aspirations and potential;
3. In a social environment, good health provides the potential to ‘negotiate’ demands of the social environment.[47]

Salutogenesis

In addition to these definitions of health, the American-Israeli medical sociologist Aaron Antonovsky, in 1979, introduced a new view on health, which he called salutogenesis, i.e. the study of generating health, as opposed to pathogenesis, the study of the origin and development of disease.[48] Antonovsky, during his qualitative studies in Israel, found that a certain group of women, who had survived concentration camps, did not have negative health outcomes in response to their experiences and were able to manage stress and stay healthy. Apparently, some people achieve good health despite their exposure to potentially disabling stress factors. Antonovsky studied the characteristics of these resilient people and found three personality traits, together forming what he called the Sense of Coherence (SOC).

The components of SOC are comprehensibility (1), manageability (2), and meaningfulness (3). The first trait refers to understanding the situation of one’s own life, the second to experiencing that one to a certain extent can influence this situation, and the third to experiencing some kind of meaningfulness in life. Antonovsky described meaningfulness as the strongest factor. In concordance with the views of psychiatrist and concentration camp survivor Viktor Frankl, Antonovsky based the importance of meaningfulness on his experiences with and observations of concentration camp survivors.[49] His approach is known as the Third Viennese School of Psychotherapy. SOC reflects two of the three previously described components in Tarlov’s analysis of definitions of health: The component of capacity to perform (1) reflects manageability, whereas the achievement of individual fulfilment (2) reflects meaningfulness. Where Antonovsky adds comprehensibility, Tarlov describes the importance of the individual’s relation to the social environment (3).

Antonovsky rejects the view of health and disease as sheer opposites; rather he describes the two as extremes on a continuum, and stresses the possibilities to strengthen SOC, in order to enhance health. His views are further elaborated, among others, by Lindström and Eriksson.[50]

Increasing criticism of the WHO definition of health in the 21st century

In 2008, Canadian Alex Jadad (MD and DPhil) initiated a global discussion using a web-based forum (blog) for which he invited participants via the British Medical Journal.[51] This blog was an attempted re-enactment of the discussion of 60 years ago that had led to the WHO definition; however, this time with the major difference of using the power of social media. Shortly after the blog, an editorial was published in The Lancet, entitled 'What is health? The ability to adapt'.[52]

At that time, the Dutch Government also felt the urgency to redefine health in a more dynamic and operational way. At the end of 2009, a two-day international expert conference was organised on 'health'. Limitations of the WHO definition were discussed and summarised as follows:

1. The absoluteness of the word 'complete', which is utopian and contributes to medicalisation;
2. The present demography of diseases which shows a transition from infectious to mainly chronic diseases that people may live with for decades. In this context, the definition declares the large majority of people as being definitively ill, without considering their ability to cope and deal with their situation;
3. The difficulty with operationalisation, as 'complete' is not measurable.

There was broad support for moving from the present static formulation of the WHO definition of health, towards a more dynamic description, based on resilience and the human ability to cope and to maintain and restore integrity, equilibrium and a sense of well-being.

Participants questioned whether a new formulation should be called a definition, because this implies set boundaries and trying to arrive at a precise meaning. They preferred the existing definition to be replaced by a concept or conceptual framework of health. A general concept, according to sociologist Blumer, would represent the characterisation of a generally agreed direction in which to look, as a reference. But operational definitions are also needed for practical life, such as for measurement purposes. The broad discussion condensed in the dynamic general concept 'Health as the ability to adapt and to self-manage, in the face of social, physical, and emotional challenges'.

Chapter 3 presents an article in the British Medical Journal, where this concept was presented.[53]

In 2012 and 2013, this general concept was further elaborated by Huber and colleagues for operationalisation into the broad operational concept of 'positive health'. This broad concept presents optional input for 'personal factors' in the ICF operationalisation. This is described in Chapter 4.

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Chapter 3

How should we define health?

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Introduction

The current WHO definition of health, formulated in 1948, describes health as “a state of complete physical, mental and social wellbeing and not merely the absence of disease or infirmity.”[1] At that time this formulation was groundbreaking because of its breadth and ambition. It overcame the negative definition of health as absence of disease and included the physical, mental, and social domains. Although the definition has been criticised over the past 60 years, it has never been adapted. Criticism is now intensifying[2,3,4,5] and as populations age and the pattern of illnesses changes the definition may even be counterproductive. The paper summarises the limitations of the WHO definition and describes the proposals for making it more useful that were developed at a conference of international health experts held in the Netherlands.[6]

Limitations of WHO definition

Most criticism of the WHO definition concerns the absoluteness of the word “complete” in relation to wellbeing. The first problem is that it unintentionally contributes to the medicalisation of society. The requirement for complete health “would leave most of us unhealthy most of the time.”[4] It therefore supports the tendencies of the medical technology and drug industries, in association with professional organisations, to redefine diseases, expanding the scope of the healthcare system. New screening technologies detect abnormalities at levels that might never cause illness and pharmaceutical companies produce drugs for “conditions” not previously defined as health problems. Thresholds for intervention tend to be lowered—for example, with blood pressure, lipids, and sugar. The persistent emphasis on complete physical wellbeing could lead to large groups of people becoming eligible for screening or for expensive interventions even when only one person might benefit, and it might result in higher levels of medical dependency and risk.

The second problem is that since 1948 the demography of populations and the nature of diseases have changed considerably. In 1948 acute diseases presented the main burden of illness and chronic diseases led to early death. In that context WHO articulated a helpful ambition. Disease patterns have changed, with public health measures such as improved nutrition, hygiene, and sanitation and more powerful healthcare interventions. The number of people living with chronic diseases for decades is increasing worldwide; even in the slums of India the mortality pattern is increasingly burdened by chronic diseases.[7]

Ageing with chronic illnesses has become the norm, and chronic diseases account for most of the expenditures of the healthcare system, putting pressure on its sustainability. In this context the WHO definition becomes counterproductive as it declares people with chronic diseases and disabilities definitively ill. It minimises the role of the human capacity to cope autonomously with life's ever changing physical, emotional, and social challenges and to function with fulfilment and a feeling of wellbeing with a chronic disease or disability.

The third problem is the operationalisation of the definition. WHO has developed several systems to classify diseases and describe aspects of health, disability, functioning, and quality of life. Yet because of the reference to a complete state, the definition remains "impracticable, because 'complete' is neither operational nor measurable".[3,4]

Need for reformulation

Various proposals have been made for adapting the definition of health. The best known is the Ottawa Charter[8], which emphasises social and personal resources as well as physical capacity. However, WHO has taken up none of these proposals.

Nevertheless, the limitations of the current definition are increasingly affecting health policy. For example, in prevention programmes and healthcare the definition of health determines the outcome measures: health gain in survival years may be less relevant than societal participation, and an increase in coping capacity may be more relevant and realistic than complete recovery.

Redefining health is an ambitious and complex goal; many aspects need to be considered, many stakeholders consulted, and many cultures reflected, and it must also take into account future scientific and technological advances. The discussion of experts at the Dutch conference, however, led to broad support for moving from the present static formulation towards a more dynamic one based on the resilience or capacity to cope and maintain and restore one's integrity, equilibrium, and sense of wellbeing.[6] The preferred view on health was "the ability to adapt and to self-manage."

Participants questioned whether a new formulation should be called a definition, because this implied set boundaries and trying to arrive at a precise meaning. They preferred that the definition should be replaced by a concept or conceptual framework of health. A general concept, according to sociologist Blumer[9], represents a characterisation of a generally agreed direction in which to look, as reference. But operational definitions are also needed for practical life such as measurement purposes.

The first step towards using the concept of "health, as the ability to adapt and to self-manage" is to identify and characterise it for the three domains of health: physical, mental, and social. The following examples attempt to illustrate this.

Physical health

In the physical domain a healthy organism is capable of "allostasis"—the maintenance of physiological homoeostasis through changing circumstances.[10] When confronted with physiological stress, a healthy organism is able to mount a protective response, to reduce the potential for harm, and restore an (adapted) equilibrium. If this physiological coping strategy is not successful, damage (or “allostatic load”) remains, which may finally result in illness.[11]

Mental health

In the mental domain Antonovsky describes the “sense of coherence” as a factor that contributes to a successful capacity to cope, recover from strong psychological stress, and prevent post-traumatic stress disorders.[12,13] The sense of coherence includes the subjective faculties enhancing the comprehensibility, manageability, and meaningfulness of a difficult situation. A strengthened capability to adapt and to manage yourself often improves subjective wellbeing and may result in a positive interaction between mind and body—for example, patients with chronic fatigue syndrome treated with cognitive behavioural therapy reported positive effects on symptoms and wellbeing. This was accompanied by an increase in brain grey matter volume, although the causal relation and direction of this association are still unclear.[14]

Social health

Several dimensions of health can be identified in the social domain, including people’s capacity to fulfil their potential and obligations, the ability to manage their life with some degree of independence despite a medical condition, and the ability to participate in social activities including work. Health in this domain can be regarded as a dynamic balance between opportunities and limitations, shifting through life and affected by external conditions such as social and environmental challenges. By successfully adapting to an illness, people are able to work or to participate in social activities and feel healthy despite limitations. This is shown in evaluations of the Stanford chronic disease self-management programme: extensively monitored patients with chronic illnesses, who learnt to manage their life better and to cope with their disease, reported improved self rated health, less distress, less fatigue, more energy, and fewer perceived disabilities and limitations in social activities after the training. Healthcare costs also fell.[15,16]

If people are able to develop successful strategies for coping, (age related) impaired functioning does not strongly change the perceived quality of life, a phenomenon known as the disability paradox.[17]

Measuring health

The general concept of health is useful for management and policies, and it can also support doctors in their daily communication with patients because it focuses on empowerment of the patient (for example, by changing a lifestyle), which the doctor can explain instead of just removing symptoms by a drug. However, operational definitions are needed for measurement purposes, research, and evaluating interventions.

Measurement might be helped by constructing health frames that systematise different operational needs—for example, differentiating between the health status of individuals and populations and between objective and subjective indicators of health. The measurement instruments should relate to health as the ability to adapt and to self-manage. Good first operational tools include the existing methods for assessing functional status and measuring quality of life and sense of wellbeing. WHO has developed several classification systems measuring gradations of health.[18] These assess aspects like disability, functioning, and perceived quality of life and wellbeing.

In primary care, the Dartmouth Cooperative Group (COOP)/Wonca (the world organisation of family doctors) assessment of functional status, validated for different social and cultural settings, has been developed to obtain insight into the perceived health of individuals. The COOP/Wonca Functional Health Assessment Charts present six different dimensions of health, each supported by cartoon-like drawings.[19,20] Each measures the ability to perform daily life activities on a 1 to 5 scale. Such instruments offer valuable information about a variety of aspects, from functioning to the experienced quality of life. Yet there are few instruments for measuring aspects of health like the individual's capacity to cope and to adapt, or to measure the strength of a person's physiological resilience. A new formulation about health could stimulate research on this.

Conclusion

Just as environmental scientists describe the health of the earth as the capacity of a complex system to maintain a stable environment within a relatively narrow range,[21] we propose the formulation of health as the ability to adapt and to self-manage. This could be a starting point for a similarly fresh, 21st century way of conceptualising human health with a set of dynamic features and dimensions that can be measured. Discussion about this should continue and involve other stakeholders, including patients and lay members of the public.

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Chapter 4

Towards operationalisation of the new dynamic concept of health, leading to 'positive health'

A shorter version is submitted for publication

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Abstract

Objective - Explorative study towards a conceptual and operational framework related to the general concept of health 'as the ability to adapt and self-manage'.
Design - A mixed methods approach was used, consisting of a qualitative study including interviews and focus groups, followed by a quantitative study – a survey – to verify the qualitative results.
Participants - The qualitative and quantitative phases involved 140 and 1938 participants, respectively, from seven main stakeholder domains of healthcare: healthcare providers (physicians, nurses, physiotherapists), patients with a chronic condition, policymakers, insurers, public health actors, citizens and researchers.
Results - The positive elements of the new concept were considered its emphasis on people being more than their illness and its focus on their strengths rather than their weaknesses. Negative elements included the concern about the concept requiring substantial personal input, as not all people were believed capable of providing such input. In the qualitative study, 556 health indicators were identified, categorised into six main dimensions of health – *bodily functions, mental functions & perception, spiritual/existential dimension, quality of life, social & societal participation, and daily functioning;* together containing 32 underlying aspects. The quantitative study found that *bodily functions* were assessed equally by all stakeholder groups, while scores for the other dimensions often differed significantly between stakeholder groups. Patients considered the six dimensions as almost equally important; thus, preferring a broad concept of health. The chronically ill among all stakeholder groups placed a higher value on the *spiritual/existential dimension* and lower one on *bodily functions*.
Conclusions - The study proposes the concept of 'positive health' to be applied to the broad concept of health, which can be evaluated subjectively in a web diagram. Further work is needed, with practical pilot studies using the web diagram and intervention tools, development of a measurement tool for population level, and a more elaborated conceptual framework.

Introduction

A new concept of health was introduced in 2011 by Huber *et al.*: 'Health as the ability to adapt and to self-manage, in the face of social, physical and emotional challenges'.[1] This new concept was proposed because the traditional WHO definition of health, which reads: 'Health is a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity'[2] was considered no longer adequate. When it was first formulated in 1948, the definition was groundbreaking, as in addition to the physical aspects, it also encompassed the mental and social aspects of health. At that time, morbidity mainly featured infectious diseases, and the availability of antibiotics in the post-war era promised great advances, while today chronic disease is much more prevalent. Furthermore, the old definition described an unattainable utopian and static state, according to which almost everybody could be considered ill, to some extent; which, thus, unintentionally enhanced medicalisation. With modern diagnostic techniques and steadily lowered cut-off points for lab values, borderline abnormal values are often measured, requiring treatment just to be 'on the safe side'. Over time, the need for a dynamic description of health that highlights the human capacity for resilience and for coping with new situations – as we are repeatedly required to do in life – was increasingly being felt.[3] Thus, the 'general concept' of health given above was developed at an international invitational conference for experts held in 2009. A concept was preferred over a definition that implies having set boundaries and precise, defined meanings. However, such a general concept also needs further operationalisation, for use in daily practice and for monitoring purposes. The research project described here is intended as a first step towards such operationalisation.

The study considered three research questions:

1. What do the various stakeholders consider to be the positive and negative elements of the new general concept of health (as described above), and which elements should be further specified?
2. What do different stakeholders consider to be indicators of health?
3. Do these indicators fit in with the general concept of health?

Objective

The overall purpose of the present study was to work towards a conceptual and operational framework related to the concept of health 'as the ability to adapt and to self-manage'. We involved seven main stakeholder domains of healthcare: healthcare providers, patients with a chronic condition, policymakers, insurers, public health actors, citizens, and researchers from various fields.

Methods

Study design and participants

A study was designed using a mixed methods approach[4]: A) a qualitative study, followed by B) a quantitative study; the latter to verify the results from the former. We involved seven main stakeholder domains of healthcare: healthcare providers (physicians, nurses, physiotherapists), patients with a chronic condition, policymakers, insurers, public health actors, citizens and researchers from different professional backgrounds.

A. Qualitative study

Participants and data collection

During an explorative study, conducted in the first 6 months of 2012, 37 qualitative (semi-structured) interviews, 10 focus groups and 3 discussion group sessions were held, involving a total of 140 people from 7 stakeholder domains. Meetings were held, after a preparatory introduction to the WHO definition and the new concept of health, where 3 questions were posed:

1. What do you consider positive about the new concept and what negative, and if you think the concept needs further specification, indicate in what respect?
2. What do you consider to be indicators of health?
3. Do these indicators fit in with the new concept of health?

Interviews were held with one or two stakeholders at a time. Focus group meetings were held with 5 to 10 participants, who met solely for the purpose of this study. In discussion groups, who met for different reasons but were willing to cooperate with this study, stakeholders were asked to answer the questions. Interviews were conducted and discussion groups were chaired by the first author, while focus groups were led by the first three authors. Meetings were generally in person, with only 5 interviews conducted by telephone. Except for those of the discussion groups, all meetings were audio-recorded, transcribed and summarised, and then sent back to the interviewees for approval. Written input was available from participants in discussion group sessions. The aim was to have a broad, representative spectrum of stakeholders in the Netherlands. The selection of organisations and people was based on the authors' knowledge about the field and the advice of healthcare opinion leaders. The distribution of contacts among the different stakeholder domains varied, because of restrictions in terms of time and budget. This resulted in the following stakeholder profiles:

- Healthcare providers: various medical specialists from university and non-university hospitals and general practitioners (15 interviews), physiotherapists (2 interviews) and nurses (1 focus group meeting);
- Patients with various chronic diseases (7 focus groups and 1 discussion group);

- Policymakers, including government officials from the Ministry of Health, Welfare and Sport (VWS), the President of the Health Council of the Netherlands (GR), the Director of the Netherlands Organisation for Health Research and Development (ZonMw) and one of its staff members, the President of the Royal Dutch Medical Association (KNMG) (7 interviews), and 6 nursing homes directors (1 discussion group meeting);
- Insurers: representatives from 1 smaller and 3 large insurance companies (4 interviews);
- Public health actors: representatives from a variety of organisations, such as the director of the Dutch Association of GGDs (Community Health Services), the team leaders responsible for the Public Health Status and Outlook report (VTV) and the project leaders responsible for the National Self-Management Action Programme (LAZ) (4 interviews and 1 discussion group meeting);
- Citizens: a group of healthy subjects from several GP practices (1 focus group meeting) and a random group of individuals over the age of 70 from an elderly network (1 focus group meeting);
- Researchers: scientists from different institutes in the fields of human physiology, stress physiology, nutrition and health, psychology and social medicine (5 interviews).

Data analysis

The first author systematically categorised the answers to Questions 1 and 3 in a qualitative way, based on content and similarities. The answers to Question 2, about the indicators of health, were coded and then categorised in several steps. Firstly, the first three authors reached consensus on the categorisation, guided by three sources: 1) clustering as produced by people in the focus groups; 2) knowledge of the context in which statements were made; and 3) the literature on existing classifications.[5,6,7,8,9] This resulted in an initial choice of categories. In a subsequent step, two experienced researchers from research institute NIVEL, independently categorised the statements, taking the proposed categories into consideration. The final step involved consensus meetings with all researchers. All differences of opinion were discussed and the main categories (the *dimensions*) and contents were agreed in consensus.
Question 3 was a closed question, requiring a yes or no answer; therefore, these responses were quantified as percentages.

B. Quantitative study

The survey

The results of the qualitative study were quantitatively tested in an anonymous, structured, online questionnaire for all stakeholder groups. Participants were asked to answer the questions from the perspective of their respective roles in healthcare, where applicable. The survey included questions on demographic and other characteristics (e.g. if participants had ever had a chronic illness). The general questions were further adapted to the sub-groups of doctors, nurses, and physiotherapists. Following an introduction about the existing WHO definition and the new concept of health, respondents were asked for their positive and/or negative opinions about the latter. The questionnaire presented the main positive and negative opinions as expressed in the interviews. Respondents could agree or disagree with an unrestricted number of opinions. They were also invited to make additional positive or negative comments. Thirty-two statements covering all health domains that were induced from the interviews were presented, and respondents were asked if they considered the statements to be related to 'health' and if so, which level of importance they would award to each statement. Respondents were asked to assign a score between 1 ('completely unimportant') and 9 ('highly important') to each of the statements. There was also a 'Don't know' option. The survey ended with a request to rank the 6 health dimensions, from most to least important.

Question 3, about the match between the indicators and the new concept of health, was not included in the survey questionnaire, as this was not considered a suitable medium for this question.

The survey was designed to take respondents a maximum of 20 minutes to fill out. It was piloted among several members from each stakeholder domain, before final distribution.

Participants and data collection

The survey was distributed over the course of a month, in the autumn of 2012, among adult representatives from the 7 stakeholder domains. Stakeholders were approached through research panels, where possible. Thus, physicians and physiotherapists were approached via the panels of their professional associations. Patients were approached via patients' associations and a validated panel of research agency Flycatcher, which also provided the representative panel of citizens. If no panels were available, participants were approached through networks within their stakeholder domains, including the nurses' association, policymakers, insurers, public health actors and researchers. Participants were invited to complete the survey questionnaire via an email containing a link to the online survey. Announcements with a link to the questionnaire were also included in newsletters, posted on the websites of various patients' associations and distributed via social media.

Data analysis

General characteristics of respondents and the responses regarding the positive and negative aspects of the new health concept are presented by mean±standard deviation for numerical variables and number (N) and/or % for categorical variables. Where a respondent indicated 'Don't know', we assigned the mean score of his stakeholder (sub-)group for this aspect, and calculated composite scores, that we compared to the composite scores with 'Don't know' as missing. Comparing these composite scores did not lead to any significant differences, and therefore we considered assigning the mean score to be appropriate. Cronbach's alphas were used to indicate the internal consistency of the survey. In addition, mean scores for each health aspect were calculated and used to construct composite scores reflecting their importance. Uncontrolled univariate general linear models (GLM) were fitted to test differences in mean composite scores between various stakeholder groups and sub-groups. Subsequently, GLM models were fitted, controlling for age, gender, level of education and chronic disease, and to indicate which variables were independently related to the dimension composite scores. Models for comparison between patients and sub-groups of healthcare providers and within these sub-groups, were not controlled for level of education, since this characteristic is strongly connected with the profession itself (i.e. academic degree for doctors, professional training for nurses and physiotherapists). For comparison between nurses with and without a chronic disease (at some time), a model was not controlled for gender, due to insufficient numbers of male nurses who had (experienced) a chronic disease. Data were analysed using SPSS 21.0 (IBM, NY, USA). A two-sided p-value ≤ 0.05 was considered statistically significant.

Results

A. Qualitative study

Question 1, 'What do you consider positive about the new concept and what negative, and if you think the concept needs further specification, indicate in what respect?' **elicited the following *positive* aspects:**

- it emphasises that a person is more than his/her illness and that besides having a chronic illness, he or she still has a large potential for being healthy;
- the focus is on a person's strength and not his/her weakness;
- it refers to self-management;
- it refers to individual responsibility;
- health is described as being dynamic, instead of a static state;
- it may make the relationship between patient and healthcare provider more equal.

Elicited *negative* aspects were:

- the concept is too broad and about life as a whole, instead of only about health;
- it denies that health is mainly the absence of disease;
- it requires substantial personal input from people – is everybody capable of this?;
- it entails personal responsibility – does everybody want this?;
- it seems to ignore the importance and impact of real illness;

- it brings the risk that people will consult a doctor too late;
- it can be an excuse for policymakers, as if people just need to adapt to the existing, poor living conditions.

Specifications that were advised:

- emphasise that this concept of health is different from health being the 'absence of disease';
- health is a domain that can be developed, publicly and privately, in addition to the domain of the medical treatment of disease and the diseased;
- emphasise that actions to enhance health should take into account people's capabilities and motivations to act;
- consider referring to the concept as 'positive health';
- visualise it, if possible, for example by using a web diagram.

Question 2, 'What do you consider to be indicators of health?', yielded 556 statements, which were categorised in a consensus process, with two independent researchers from the NIVEL institute, into 6 dimensions, covering 32 aspects, and a small diverse 'other' group, including healthy behaviours and environmental factors. Table 1 shows the 6 dimensions of health and the related 32 aspects.

Table 1 The 6 dimensions of the health indicators, covering 32 aspects of health

Bodily functions	**Mental functions & perception**	**Spiritual/ existential dimension**	**Quality of Life**	**Social & societal participation**	**Daily functioning**
Medical facts	Cognitive functioning	Meaning/ meaning-fulness	Quality of life/ well-being	Social and communicative skills	Basic ADL
Medical observations	Emotional state	Striving for aims	Happiness	Social contacts	Instrumental ADL
Physical functioning	Esteem/ self-respect	Future prospects	Enjoyment	Meaningful relationships	Ability to work
Complaints and pain	In control/ manageability	Acceptance	Perceived health	Being accepted	Health literacy
Energy	Self-management		Flourishing	Community involvement	
	Resilience, SOC (sense of coherence)		Zest for life	Meaningful work	
			Balance		

Question 3, 'Do these indicators fit in with the new concept of health?'. Overall, 78% of responses were positive, 12% negative and 10% hesitant, thus showing ample support for the match between the concept and the indicators among individual stakeholders.

B. Quantitative study

Demographic characteristics

An overview of the characteristics of the 1938 respondents from the 7 stakeholder domains is presented in Table 2.

More than half of all respondents had experienced at least one chronic condition in his/her life. Cardiovascular disease (15.7%), neck and/or backache (13.6%), diabetes (11.3%), chronic joint problems (10.7%), respiratory disease (10.3%), and cancer (5.7%) were the most common chronic conditions.

Positive and negative aspects of the new concept

The 1938 respondents scored 4514 positive and 3443 negative aspects, plus an additional 4 positive and 5 negative aspects in the category 'other'. (Tables 3 and 4)

Mean scores and composite scores per health aspect *(See Table I in Supplement)*

The mean scores for each of the 32 questions varied from 6.58 to 7.80 on a 9-point scale with standard deviations ranging from 1.13 to 1.99. Relatively higher scores were mainly allocated to aspects associated with *bodily functions* and *quality of life*. Aspects associated with the *spiritual/existential* dimension and *social & societal participation* were given relatively lower scores.

Subsequently, composite scores were calculated, based on the sub scores on corresponding health aspects. Cronbach's alphas were calculated as a measure of the internal consistency of the survey's health aspects and its composite scores. The results are summarized in Table 5.

Cronbach's alphas were just above or below 0.90 for 5 of the 6 dimensions, which indicates that the mean aspect scores were strongly correlated within these dimensions. The Cronbach's alpha for *bodily functions* was lower. Deletion of one of the aspects did not lead to a higher score. However, a Cronbach's alpha of 0.70 for this dimension is still considered acceptable. Overall, the results demonstrate that, for each dimension, one is justified in interpreting a composite score that has been aggregated from its aspect scores.

Table 2 General characteristics of respondents

	Total	Healthcare providers	Patients	Citizens	Policymakers	Insurers	Public health actors	Researchers
N	1938	643	575	430	80	15	89	106
%	100	33.2	29.7	22.2	4.1	0.8	4.6	5.5
Gender (%)								
Male	42.5	43.3	41.2	51.2	31.3	73.3	19.1	33.0
Female	57.5	56.6	58.8	48.8	68.8	26.7	80.9	67.0
Age (years ± SD)	51.6±13.4	50.3±9.5	56.6±12.7	52.5±16.6	44.6±10.9	42.6±10.1	45.1±11.5	40.6±11.3
Type of employment (%)								
Independent	16.9	41.8	5.6	5.3	0	0	1.1	2.8
Salaried	50.0	52.9	28.3	44.7	98.8	100.0	95.5	89.6
Flexible / on call	2.0	0.6	2.4	4.9	0	0	0	0
Retired	14.3	1.6	29.4	22.6	0	0	1.1	0.9
Unable to work / disability (full or partial)	5.2	0	17	0.2	0	0	0	0.9
Social security benefit	0.6	0	1.4	0.5	1.3	0	0	0
No work/other	9.1	0.6	14.4	20.7	0	0	1.1	0
Combination of jobs	1.9	2.5	1.4	1.2	0	0	1.1	5.7
Highest level of education (%)								
Elementary or high school	6.2	0	10.6	13.3	2.5	0	0	0
Junior vocational training	4.6	0	9.9	7.7	0	0	0	0
Secondary vocational training	23.2	4.2	40.7	42.6	0	0	4.5	0.9
Professional training	29.8	46.5	28.2	21.9	5	26.7	13.5	1.9
University degree	36.2	49.3	10.6	14.7	92.5	73.3	82.0	97.2
Personal experienced disease(s) (%)								
Yes	55.3	33.3	100	48.1	26.3	20.0	29.2	24.5
No	44.7	66.7	0	51.9	73.8	80.0	70.8	75.5

Table 3 Positive aspects of the new health concept according to respondents

I feel positive about the new concept **:	**N**	**%***
It emphasises that a person is more than his/her illness	967	49.9
It emphasises people's strengths rather than their weaknesses	943	48.7
Health is seen as a dynamic rather than a static state	876	45.2
It emphasises self-management	862	44.5
It focuses on individual responsibility	581	30.0
It makes the patient more equal to the practitioner	285	14.7
Other (summarised):		
It focuses attention on the path towards health	12	0.6
It is broad and feasible	10	0.5
It stresses that you can still feel healthy despite a chronic disease	8	0.4
It emphasises adaptability	6	0.3
Other / don't know / nothing negative	83	4.2

** % of the total population (N=1938)*
*** respondents were free to give more than one answer*

Table 4 Negative aspects of the new health concept according to respondents

I feel negative about the new concept **:	**N**	**%***
It asks a lot from people, is everyone capable of self-management?	810	41.8
This description seems to make actual disease unimportant	604	31.2
This is too broad, it is about life and not about health	599	30.9
For me, health is primarily the absence of disease	426	22.0
It asks a lot from people, does everyone want to take responsibility?	426	22.0
Should anyone simply adjust to poor living conditions?	337	17.4
It could keep someone from going to the doctor in time	241	12.4
Other (summarised):		
It is vague and unclear	32	1.7
Not everything can be attributed to individual behaviour	19	1.0
Too little consideration of the influence of the social environment	16	0.8
There is a danger of victims being blamed	13	0.7
Old definition is fine	10	0.5
Other / don't know / nothing positive	95	4,9

** % of the total population (N=1938)*
*** respondents were free to give more than one answer*

Table 5 Cronbach's alphas per health dimension

Dimension composite score	Number of aspects	Cronbach's Alpha
Bodily functions	5	0.70
Mental functions & perception	5	0.89
Spiritual/existential dimension	5	0.92
Quality of life	7	0.93
Social & societal participation	6	0.94
Daily functioning	4	0.88

Importance of health dimensions as viewed by stakeholders

General

Mean composite scores about the importance per dimension as being part of 'health' are represented on a 9-point scale in Figure 1, per stakeholder group.

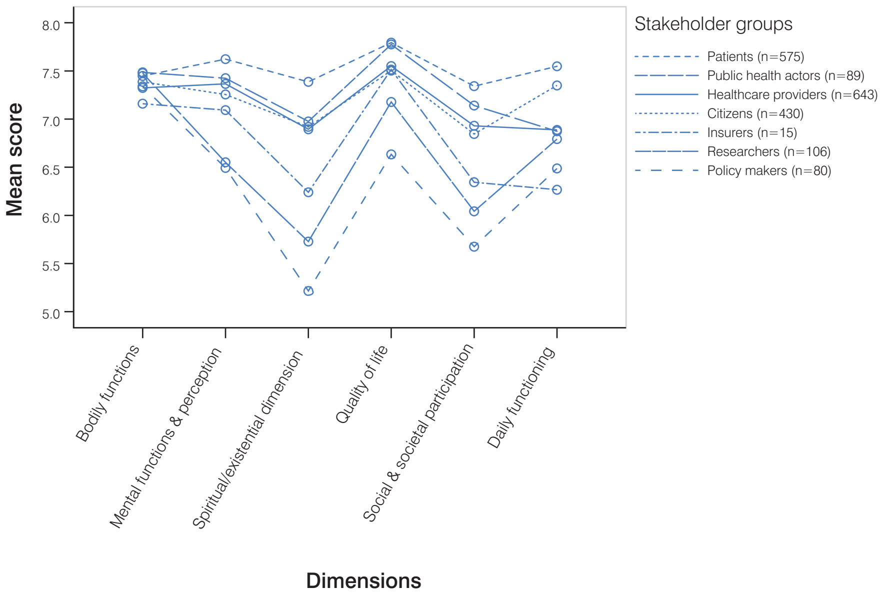

Figure 1 Mean scores per stakeholder group on a 9-point scale, indicating the importance assigned by respondents to the dimensions as being part of 'health'

The figure shows marked variation in mean composite scores between all stakeholder groups within each health dimension, except for *bodily functions.* Uncontrolled GLM confirmed a non-significant difference between stakeholder groups for *bodily functions* (p= 0.583), in contrast to significant differences between stakeholder groups for all other dimensions (all p-values ≤ 0.001). GLM showed that these differences were still significant after controlling for age, gender, level of education, and chronic disease (p= 0.628 for *bodily functions*, p=0.040 for *daily functioning*, and p<0.001 for all other dimensions).

Patients and healthcare providers *(See Table II in Supplement)*

Figure 2 shows the mean composite scores on the importance assigned to each dimension as part of 'health' by healthcare providers (subdivided into physicians, nurses and physiotherapists) and patients.

The mean composite scores of the physicians were generally the lowest. Uncontrolled GLM demonstrated that all mean composite scores between patients and physicians

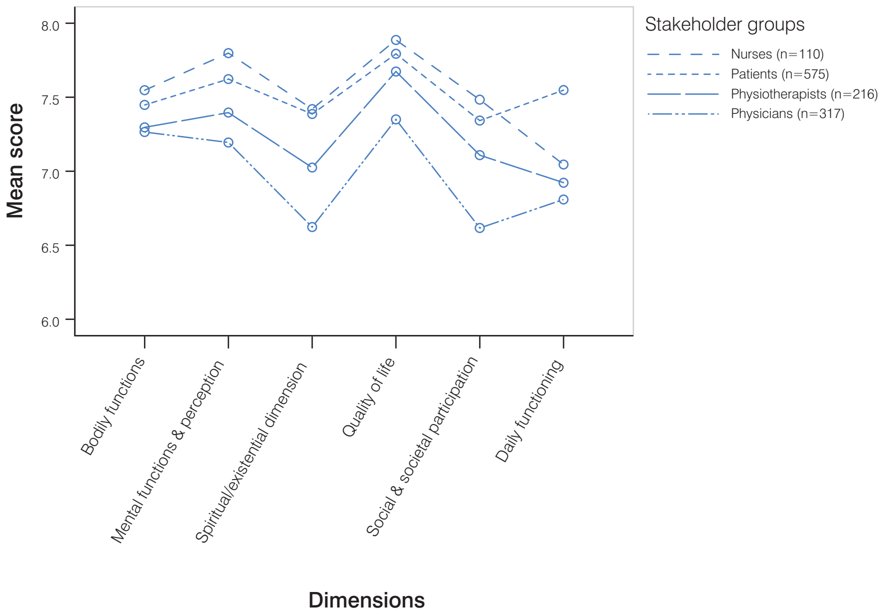

Figure 2 Mean scores per healthcare provider group and patients on a 9-point scale, indicating the importance assigned by respondents to the dimensions as being part of 'health'

were significantly different (all p-values ≤ 0.042). In contrast, the mean composite scores of the nurses were generally the highest, and most congruent with those of patients (all p-values ≥ 0.105) except on *daily functioning* (p=0.002). After controlling for age, gender, and chronic disease, all differences in dimension composite scores between physicians and patients remained statistically significant (all p-values ≤ 0.034), except for the dimension of *bodily functions* (p=0.136). Furthermore, after controlling for age, gender, and chronic disease, the dimension composite scores of the nurses were significantly higher than those of the patients, in the cases of *mental functions & perception* (p= 0.007), *spiritual/existential* dimension (p= 0.012) and *social & societal participation* (p= 0.003). This change in significance after controlling was caused by the effect of both age and chronic disease. Between patients and physiotherapists, the difference for the dimension of *daily functioning* was the only one that remained significant after controlling (p= 0.018).

Healthcare providers according to whether they have experienced a chronic disease *(See Table III in Supplement)*

Uncontrolled GLM demonstrated that composite scores for physicians who had experienced a chronic disease were significantly higher for the *spiritual/existential* dimension, *social & societal participation* and *daily functioning* (all p-values ≤ 0.050), compared to physicians without experience of chronic disease. Physiotherapists with or without any experience of chronic disease did not have significantly different composite scores (all p-values ≥ 0.062). The same applied to the nurses, except for the *spiritual/existential dimension,* which was significantly higher for nurses with a

Table 6 Additional determinants for dimension composite scores

	Age			Gender*		
Dimension	**B**	**%CI**	**p**	**B**	**%CI**	**p**
Bodily Functions	0.004	0.000 – 0.007	0,068	-0.208	-0.306 – -0.109	<0.001§
Mental functions & perception	0.023	0.019 – 0.028	<0.001§	-0.165	-0.283 – -0.048	0.006§
Spiritual/existential dimension	0.026	0.021 – 0.031	<0.001§	-0.295	-0.433 – -0.157	<0.001§
Quality of life	0.012	0.008 – 0.017	<0.001§	-0.210	-0.315 – -0.106	0.001§
Social & Societal participation	0.025	0.020 – 0.031	<0.001§	-0.219	-0.357 – -0.080	0.002§
Daily functioning	0.019	0.013 – 0.024	<0.001§	-0.055	-0.188 – 0.077	0.412

* Reference category is 'female'
** Reference category is 'completed a university degree'
*** Reference category is 'without chronic disease'
§ Significant (p≤ 0.05)

chronic disease (p= 0.042). After controlling for age and gender (physicians and physiotherapists) and age (nurses) by GLM, the following significant associations were found:

- Physicians with a chronic disease considered the *spiritual/existential* dimension (p= 0.007) and the dimension *social & societal participation* (p= 0.002) significantly more important than those without a chronic disease.
- Nurses with a chronic disease considered the dimension *mental functions & perception* (p= 0.027) and the *spiritual/existential* dimension (p= 0.010) significantly more important than those without a chronic disease.
- Physiotherapists with a chronic disease considered the dimensions *bodily functions* (p= 0.017), *mental functions & perception* (p= 0.021), and *social & societal participation* (p= 0.039) significantly less important than those without a chronic disease.

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The change in significance after controlling within the sub-groups of nurses and physiotherapists was caused by the effect of age.

Additional determinants of health dimension composite scores

GLM analysis also revealed which other characteristics – in addition to 'stakeholder group' – were independently related to the dimensions' composite scores. The adjusted mean differences between groups, indicated by the regression coefficient B, with the corresponding 95% confidence intervals (CI), are presented in Table 6, showing that an increase in age was independently related to higher levels of

	Level of education (university versus non-university) **			Having a chronic disease ***		
Dimension	B	%CI	p	B	%CI	P
Bodily Functions	0.061	-0.062 – 0.184	0.330	-0.140	-0.259 – -0.021	0.021§
Mental functions & perception	0.369	0.223 – 0.516	<0.001§	0.045	-0.097 – 0.187	0.535
Spiritual/existential dimension	0.647	0.476 – 0.818	<0.001§	0.170	0.003 – 0.336	0.046§
Quality of life	0.412	0.282 – 0.542	<0.001§	0.043	-0.083 – 0.170	0.502
Social & Societal participation	0.721	0.548 – 0.893	<0.001§	0.116	-0.052 – 0.283	0.177
Daily functioning	0.275	0.111 – 0.440	0.001§	0.046	-0.114 – 0.206	0.577

perceived importance within each dimension. Each year, the perceived importance of the dimensions' composite scores increased by the magnitude of regression coefficient B. Furthermore, being male resulted in significantly lower composite scores for all dimensions, except *daily functioning.* In addition, having a chronic disease was independently related to lower scores for the dimension of *bodily functions* and higher scores for the *spiritual/existential* dimension. Exploration of the data revealed that people who had completed a university degree (regardless of their stakeholder group) tended to have lower composite scores compared to people of all other levels of education. This characteristic was therefore divided into two levels: completion of a university degree, and all other levels of education. Having completed a university degree was independently related to lower composite scores for all dimensions, except *bodily functions*.

Discussion

In this exploratory study, we took the first steps in developing a conceptual and operational framework, related to the concept of health 'as the ability to adapt and to self-manage', by involving seven most important stakeholder domains in health – healthcare providers, patients with a chronic condition, policymakers, insurers, public health actors, citizens and researchers – in a qualitative and quantitative study based on the Netherlands.

The results from the qualitative study showed that most respondents from all stakeholder domains appreciated the newly devised concept, because it emphasised that people are more than their illness and itconsiders their strengths instead of their weaknesses. They also valued that the new concept described health as dynamic and referred to personal self-management and responsibility. Most respondents felt negative about the fact that the concept requires substantial personal skills and input for realising adequate adaptation and self-management, and they wondered whether all people would be capable of providing such input. Moreover, respondents felt the description seemed to ignore any current disease or possibly underestimated its importance. A minority of respondents felt the concept was too broad and not about health but about life as a whole. These opinions were confirmed in the quantitative survey. Specific suggestions included: 'emphasise that this concept is different from and more than "health as the absence of disease"'; 'consider calling it "positive health" and visualising it, for example, by using a web diagram'; and 'emphasise that health is a domain that can be developed, if people's capabilities and motivations are taken into account'.

The study also identified, in a consensus process, 6 main dimensions of health: *bodily functions, mental functions & perception, spiritual/existential* dimension*, quality* *of life, social & societal participation,* and *daily functioning*, and 32 underlying aspects of health.

In the quantitative survey the health dimensions and underlying aspects, as inducted by the qualitative study, were tested for their perceived importance for 'health', among larger groups from the 7 stakeholder domains. All stakeholder groups regarded the dimension of *bodily functions* as equally important. The evaluation of the other 5 dimensions showed significantly different valuations by the respondents. The highest score was for *quality of life,* closely followed by *bodily functions*. Aspects belonging to the *spiritual/existential* dimension and to *social & societal participation* were given relatively lower scores and, in addition, showed stronger variation between different groups of stakeholders. Patients considered all 6 dimensions as almost equally important, while other stakeholder groups often gave significantly different scores for various dimensions. Healthcare providers differed significantly from patients. However, when the group was divided into physicians, physiotherapists and nurses, the nurses appeared to assess matters almost identically to patients. Citizens (the general population) also differed from patients. Having experienced a chronic disease was itself independently related to a decrease in the value placed on *bodily functions* and to an increase in the value accorded the *spiritual/existential* dimension*.* The other 4 dimensions were not independently related to chronic disease, but rather to advancing age, gender and level of education. This association is still interesting, as the onset of a chronic disease is usually related to advancing age, and patients generally have a relatively lower level of education than their healthcare providers.
The impact of chronic disease for the interpretation of health was confirmed by the shift in the perceived importance of several health dimensions, if healthcare providers had experienced a chronic disease themselves. For physicians who had at some time or other experienced a chronic disease, the *spiritual/existential* dimension and *social & societal participation* become more important, and for nurses *mental functions & perception* and the *spiritual/existential* dimension rose in importance*,* while for both groups *bodily functions* became less important. For physiotherapists who had experienced a chronic disease, there was also a change in valuation, as *bodily functions, mental functions & perception* and *social & societal participation* became less important.

Several limitations of the study need to be considered. The first question, concerning positive and negative aspects and the need for specification, seemed uncontroversial, but in the selection of health indicators, it may be argued that the study design was too open and explorative. Why not opt for operationalization by existing questionnaires about coping, resilience and self-management – aspects that clearly connect to the new concept? Our reason for choosing an open approach was that

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the general concept itself is the result of a process whereby a wide variety of characterisations of health, as expressed by participants at the invitational conference in 2009, were gradually condensed into a concept. We wanted to respect the expected broad range of opinions among the main stakeholder domains in healthcare, by also exploring the question about indicators of health by an inductive approach, as described in 'grounded theory'.[102] This resulted in the bottom-up categorisation of 6 main dimensions and 32 aspects of health.

In the interviews, different stakeholders, such as medical specialists, patients and policymakers, described different indicators and indicated whether each of these self-defined indicators matched the new concept (question 3). However, in the survey, the categorised indicators were presented to all stakeholders and we considered the opinions of the different stakeholders about whether certain aspects and dimensions are part of 'health' to be more relevant than yes/no answers about a match between all indicators and the concept. So we did not include question 3 in the survey. This fact could be considered a limitation of the study.

The study was conducted in the Netherlands and its generalisability to other, especially non-western populations will need to be studied.

A strength of the present study is its large number of respondents: 300–575 in the panels and 80–106 in networks of stakeholders, except for the insurers, with only 15 willing to participate. Concerning the representativeness of the data, the panels from the research agency were validated for the Dutch population. The participants of the other panels and networks could have been selected from the more active part of the population. However, as this concerned several groups, any influence on the contrast in the results is not expected.

Another strength of the study lies in the fact that the content of the specific domains was induced bottom-up from 556 expressed indicators in a consensus process involving experienced researchers. Cronbach's alphas were acceptable for the dimension of *bodily functions* and good to excellent for all other dimensions, which confirms that the identified health dimensions and their underlying aspects were consistent in terms of composition.

The existing literature about health indicators was studied, but statements in the qualitative phase covered a broader area of life than most generic measures of perceived health and health-related quality of life. The fact that we found such broadness might be considered a weakness, as we cannot connect the results to an existing measurement instrument. However, it might be considered a strength that our results have a broad empirical basis. We found most congruence of our findings with the dimensions and aspects reported in a study by Stewart *et al.*[9] on the quality of life of dying persons, and as described by Willemstein *et al.*[10] for outcome measures in the care sector (in Dutch). The WHO's International Classification of Functioning, Disability and Health (ICF) is a respected and widely accepted terminology for health, functioning, and health-related domains with multi-dimensional concepts. However, we were not able to categorise and code many of the statements using the present ICF classifications. One important reason for this is that the ICF lacks a classification of personal factors which many of our aspects seemed to suit best. The first and fifth author – an ICF specialist and researcher with a focus on the category of 'personal factors'[11] – compared the 6 dimensions and 32 aspects in this study against the ICF classes. For this purpose, the aspects were linked to the most appropriate ICF categories using the linking rules devised by Cieza *et al.*[12] Of the 32 aspects, 18 were coded as personal factors. We concluded that the personal factors found in our study could be used as input for attempts to formulate a list of ICF Personal Factors, while available instruments could be selected for measuring several of our identified aspects of health, based on a link with the appropriate ICF codes.

Having a chronic disease was itself independently related to a decrease in the value placed on *bodily functions* and to an increase in the value accorded the *spiritual/ existential* dimension. This phenomenon – a change in perception after a major life event such as facing a chronic disease – seems to connect with what is described as a transformational event or the phenomenon of 'response shift'. This is a change in experienced quality of life and health-related quality of life when facing a serious illness, which has been described in general terms and in specific diseases.[13,14,1516,17]

The discrepancy between the broad perception of health as indicated by the stakeholder group of patients who evaluated all six dimensions as almost equally important, and the quite narrow perception of health (main emphasis on *bodily functions* and *quality of life*) indicated by participants in most other stakeholder domains, including citizens in healthcare, warrants attention. The results would suggest that several common cost-utility tools, such as the Global Burden of Disease (GBD)[18] and the Quality Adjusted Life Year (QALY)[19], should not take citizens or healthcare workers as a reference. Our results suggest that subjects with a disease experience their burden of disease, as well as their various health dimensions and quality of life, quite differently from healthy subjects.

Some respondents feared that the new concept might overlook current diseases or underestimate their importance. It should be emphasised that we did not in any way underestimate the major importance of the domain of curative healthcare. Yet, we stress the urgency of enhancing health promotion because, similar to many other Western countries, the Netherlands spends 96% of its national healthcare budget on cure and care and as little as 4% on health promotion.[20]

In the Netherlands, the prevailing trend in policy is 'patient-centred care'. However, if this is to be taken seriously, patients' broad perception of health should receive much more attention from policymakers. Several respondents felt that the framing of the word 'health' in the broad context of the 6 dimensions should be specified, in order to prevent confusion with the common understanding of health being 'the absence of disease'. Some respondents suggested adopting the phrase 'positive health' in this context, indicating that it precedes even primary and secondary prevention and aims for health development in a general sense. This concept can already be found in the literature[21,22,23,24], used in the sense of health promotion, albeit rather vague and in a slightly different context.

Another recommended specification concerned visualisation of the broad concept of health for practical use; for example, in a web diagram. The web diagram could be used in conversations between health professionals and clients/patients, suggesting possible topics for subjective evaluation and, if desired, potential actions for improvement. A possible visualisation is presented in Figure 3. A similar approach already proved to be a useful tool in communication between care providers and patients in mental health services[25].

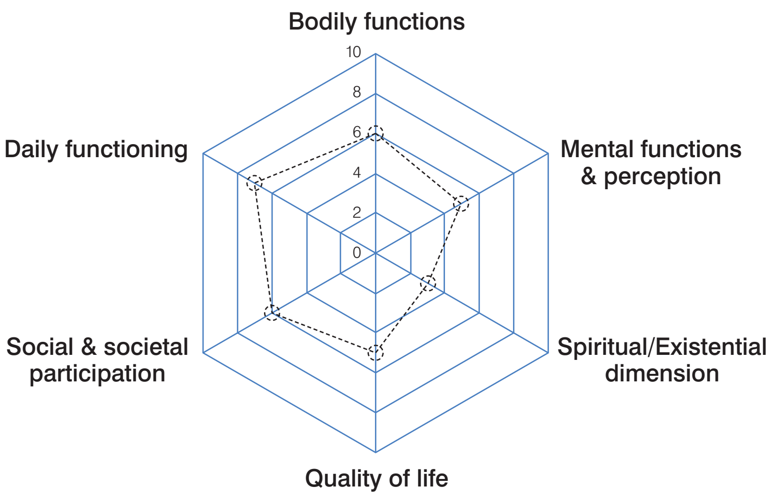

Figure 3 The 6 dimensions on a subjective scale, visualised for practical use, representing a fictional estimation of a person's state of 'positive health'

Combining the two recommendations described above, we propose the concept of 'positive health', connected to the 6 dimensions of health, and the web diagram as a first 'definitive concept', in the sense of Blumer[4], which has been elaborated from 'Health as the ability to adapt and to self-manage'.[1]

We emphasise that our study focused on the indicators of health and not on determinants. Yet, in the focus groups, many patients stated that the indicators they mentioned were also determinants of health. Future research should explore whether interventions on aspects of the various domains result in improvements in physical health, in functioning in the different dimensions and/or in health-related quality of life. Future research should also select or develop measurement tools for the various domains and aspects, in order to create an objective instrument.

In future elaborations on the conceptual framework, the external factors that undeniably influence a person's ability to adapt and to self-manage, such as the social, political, economic and environmental factors, should also be described, as is emphasised by Shilton *et al.* of the International Union for Health Promotion and Education (IUHPE).[26]

We conclude that patients interpreted the new dynamic concept of health as one that encompasses life as a whole, and that if health professionals had experienced disease themselves, their value system also shifted towards an increased appreciation of aspects such as meaningfulness, while physical aspects became less important as part of health. This finding warrants reflection on the content of medical training, as well as on medical practice.

Supplement

Table I Mean scores per health aspect (excluding the answer category of ´Don’t know´)

Aspects per dimension	N	Mean ± SD*	‘Don’t know’
1.1 ... a medical check shows no abnormal results	1922	7.07±1.86	16
1.2 ... giving a healthy impression (colour/eye expression/ attitude)	1924	6.75±1.78	14
1.3 ... having an age-appropriate physical capacity to function	1927	7.67±1.26	11
1.4 ... not being plagued by complaints of pain	1921	7.59±1.41	17
1.5 ... feeling energetic	1923	7.77±1.22	15
Total of dimension 1: Bodily functions	**1897**	**7.37±1.03**	**41**
2.1 … being mentally competent and able to think clearly	1923	7.69±1.45	15
2.2 ... being in a positive mood	1932	7.14±1.59	6
2.3 ... having self-confidence	1929	7.00±1.68	9
2.4 ... having a grip on his/her life	1928	7.41±1.49	10
2.5 ... being able to manage personal circumstances.	1925	7.38±1.53	13
Total of dimension 2: Mental functions & perception	**1910**	**7.32±1.30**	**28**
3.1 .. .being able to find meaning in life	1908	6.80±1.83	30
3.2 ... being able to do fulfilling activities	1913	6.90±1.77	25
3.3 ... having ideals and live for them	1917	6.58±1.94	21
3.4 ... having confidence in the future	1918	6.88±1.81	20
3.5 ... being able to accept and be satisfied with life	1920	7.21±1.74	18
Total of dimension 3: Spiritual/existential dimension	**1882**	**6.87±1.58**	**56**
4.1 ... experiencing a good quality of life	1927	7.71±1.24	11
4.2 ... feeling happy most of the time	1929	7.28±1.46	9
4.3 ... being able to enjoy life	1931	7.42±1.52	7
4.4 ... feeling healthy	1932	7.80±1.13	6
4.5 ... flourishing	1930	7.62±1.31	8
4.6 ... having a zest for life	1933	7.59±1.33	5
4.7 ... being well-balanced	1926	7.45±1.46	12
Total of dimension 4: Quality of life	**1914**	**7.56±1.13**	**24**
5.1 ... being able to maintain social contacts	1929	7.07±1.63	9
5.2 ... having sufficient supportive relationships	1928	6.91±1.73	10
5.3 ... not experiencing loneliness	1932	7.03±1.72	6
5.4 ... feeling accepted in the social environment	1928	7.00±1.74	10
5.5 ... participating in society	1925	6.77±1.75	13
5.6 ... doing work (paid or unpaid) that is perceived as meaningful	1916	6.73±1.99	22
Total of dimension 5: Social & societal participation	**1900**	**6.92±1.56**	**38**
6.1 ... being able to wash and dress oneself (basic ADL)	1927	7.54±1.52	11
6.2 ... being able to run the personal household (e.g. cooking, cleaning, managing money)	1930	7.20±1.59	8
6.3 ... being able to work (paid or unpaid)	1925	6.87±1.78	13
6.4 ... being able to understand medication instructions and follow them (health literacy)	1923	6.95±1.80	15
Total of dimension 6: Daily functioning	**1916**	**7.14±1.43**	**22**

* SD=Standard Deviation

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Table II Mean composite scores for healthcare providers compared to patients

	Patients (N=575)	Physicians (N= 317)		
	Mean±SD*	Mean±SD*	p**	p controlled.***
Bodily Functions	7.40 ±1.10	7.26±0.98	0.042§	0.136
Mental functions & perception	7.59 ±1.19	7.19±1.28	<0.001§	0.034§
Spiritual/existential dimension	7.33 ±1.35	6.58±1.58	<0.001§	0.003§
Quality of Life	7.77 ±1.05	7.34±1.16	<0.001§	0.009§
Social & societal participation	7.31 ±1.44	6.61±1.53	<0.001§	0.005§
Daily functioning	7.52 ±1.34	6.81±1.43	<0.001§	<0.001§

* SD= Standard Deviation

** Uncontrolled p-value for difference in mean score between patient and specific healthcare provider, as determined by GLM

*** p-value for difference in mean score between patient and specific healthcare provider, controlled for age, gender, chronic disease, as determined by GLM

§ Significant ($p \leq 0.05$)

Table III Mean composite scores for healthcare providers with and without (having faced) a chronic disease

	Physicians (N= 317)			
	With chronic disease (N=122)	Without chronic disease (N=195)	p**	p controlled.***
	Mean±SD*	Mean±SD*		
Bodily Functions	7.25 ±0.97	7.26±0.99	0.943	0.966
Mental functions & perception	7.35 ±1.18	7.09±1.33	0.071	0.152
Spiritual/existential dimension	6.94 ±1.31	6.36±1.69	0.001§	0.007§
Quality of Life	7.51 ±0.99	7.23±1.24	0.036§	0.062
Social & societal participation	7.00 ±1.24	6.37±1.65	<0.001§	0.002§
Daily functioning	7.00 ±1.40	6.68±1.44	0.055	0.116

* SD= Standard Deviation

** Uncontrolled p-value for difference in mean score between specific healthcare providers who have (had) a chronic disease and those who have not, as determined by GLM.

*** p-value for difference in mean score between specific healthcare providers who have (had) a chronic disease and those who have not, controlled for age and gender, as determined by GLM.

**** p-value for difference in mean score between nurses who have (had) a chronic disease and nurses who have not, controlled for age, as determined by GLM

§ Significant ($p \leq 0.05$)

Nurses (N=110)			Physiotherapists (N=216)		
Mean±SD*	p**	p controlled***	Mean±SD*	p**	p controlled***
7.54±0.88	0.205	0.735	7.29±0.97	0.153	0.331
7.79±0.87	0.105	0.007§	7.39±1.06	0.032§	0.924
7.42±1.10	0.526	0.012§	7.00±1.21	0.003§	0.301
7.89±0.84	0.276	0.076	7.67±0.86	0.242	0.342
7.48±1.06	0.224	0.003§	7.10±1.08	0.068	0.110
7.04±1.54	0.002§	0.124	6.90±1.48	<0.001§	0.018§

4

Nurses (N=110)				Physiotherapists (N=216)			
With chronic disease (N=41)	Without chronic disease (N=69)	p**	p controlled****	With chronic disease (N=51)	Without chronic disease (N=165)	p**	p controlled***
Mean±SD*	Mean±SD*			Mean±SD*	Mean±SD*		
7.60±0.79	7.50±0.93	0.563	0.466	7.11±0.98	7.34±0.96	0.141	0.017§
7.91±0.73	7.72±0.94	0.264	0.027§	7.16±1.26	7.47±0.99	0.074	0.021§
7.70±0.89	7.26±1.19	0.042§	0.010§	6.87±1.45	7.05±1.13	0.347	0.084
8.00±0.72	7.82±0.90	0.277	0.133	7.51±0.87	7.72±0.85	0.118	0.062
7.62±1.02	7.39±1.09	0.281	0.097	6.87±1.31	7.18±0.99	0.076	0.039§
7.09±1.59	7.00±1.52	0.782	0.684	6.57±1.78	7.01±1.36	0.062	0.057

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Part II

NUTRITION and HEALTH

Chapter 5

A short introduction on human nutrition and methods for evaluating the health effects from nutrition

Human nutrition through the ages

In their basic textbook on Human Nutrition and Dietetics, Davidson and Passmore[1] describe how, from a nutritional point of view, humankind can be divided into four types:

1. Primitive hunter-gatherers, covering 99% of the total period of human existence;
2. Peasant farmers and pastoralists, going back approximately 9,000 years, starting with primitive agriculture in Mesopotamia;
3. Urban slum dwellers, who usually consume food of poor value; they grew in large numbers in the 19th century, during the industrial revolution, and lived around factories. But today they are found in and near the large cities of mainly non-Western countries, but also as lower classes in Western society;
4. The mainly modern phenomenon of people in the middle and higher classes of contemporary society, who are free of the fear of crop failures and can afford to eat their favourite dishes all year round.

It is a modern development that peasant farmers are rapidly decreasing in numbers, together with a strong shift in people moving to the large cities, where they either prosper or become urban slum dwellers.

It is obvious that prosperity benefits health, which expresses itself in the growth and increasing longevity of more prosperous populations, and is caused, among other things such as good housing and education, by the availability of food.

Yet, it is also clear that an increased consumption of meat, animal fat, sugar and salt (things that are often connected to increased wealth), is not equivalent to health, even more so because this dietary pattern is often accompanied by a lifestyle of little physical activity combined with cigarette smoking and alcohol consumption. Thus, although people do get older, they increasingly also attract one or more chronic diseases, which in 50% of cases is caused by lifestyle factors.[2] However, getting older does not necessarily involve chronic disease, as is indicated by the phenomenon of co-called 'blue zones': areas in the world where people live to a ripe old age while continuing to function well for very long, without serious illnesses.[3]

Sufficient supply of healthy and sustainable food for the growing world population?

In our present time, the question of whether a sufficient level of food production to feed a fast growing world population could be achieved in a sustainable way, is becoming an urgent one. The world population is expected to grow to 9 billion people by 2050. Various agricultural production systems are used in search of the answer to this question. The number of small farms is declining, whereas today's large industrialised farms are increasing in numbers and size, all over the world. Next to this conventional type of agriculture, other types of crop production are also being developed. Organic agriculture, which involves growing food in an ecosystem situation and therefore avoiding artificial fertilisers and synthetic pesticides, is increasing in overall volume, at both small and larger farms. In addition, forms of High Tech Agriculture (HTA), which uses hydro-culture (hydroponics), LED-illumination and sensor technology to guide plant development in sterile surroundings to avoid having to use pesticides, are being developed in highly developed countries, especially in the Netherlands.[4]

Connected to these fundamentally different types of agro-production is fierce debate, often charged with emotion, on environmental sustainability, the ability to feed the world, and economic feasibility. The question of supplying sufficient amounts of food to feed the future world population is of such eminent importance that the UN has appointed a 'Special Rapporteur on the right to food'. This rapporteur, Olivier De Schutter, recently stated that 'A new paradigm on well-being, resilience and sustainability must be designed to replace the productivist paradigm and thus better support the full realisation of the right to food. The equation is complex, but is one that can be solved'.[5] This statement underlines the relevance of scientific research on a variety of production systems, including their health implications.

The following question therefore arises: How can research be designed to study the nutritional value of the foods that are produced using different production systems? What is the historical context and what are the underlying paradigms of modern nutritional science?

The development of nutritional science and some opposing views on nutrition

Nutrition is a basic condition for living, but it is also an important source to either support or damage health. This has long been known by humankind. Hippocrates, who identified illness in his patients as an imbalance in four humours, gave dietary advice as the first option to restore this balance. He identified various qualities of food products connected to these humours, and by supplementing certain products or by subscribing certain herbs he aimed to strengthen what was weak. This qualitative perception persisted for centuries, but was subsequently abandoned parallel to the paradigm change in medical thinking between the 16th and 19th century (as described in Chapter 2).

The French chemist Lavoisier is considered the founder of modern nutritional science, as he induced the change from a qualitative to a quantitative way of thinking. Although he died young – by the guillotine during the French revolution – he performed crucial experiments which inspired his successors. He experimentally demonstrated the basic law of metabolism, by demonstrating the utilisation of oxygen and production of carbon dioxide and heat when organic matter such as food is burned in a calorimeter. Later, it was shown that oxygen consumption and heat production increase after food consumption as well as during exercise. Furthermore, he identified the elements oxygen and hydrogen.[6]

In the 19th century, nutritional science gradually developed further by observational and experimental research. In 1827, William Prout differentiated between carbohydrates, protein and fats in foods, and in 1860 Claude Bernard discovered the synthesis of fat from carbohydrates and protein in the human body. The concept of the 'calorie' was first used in France in the context of heating engines, but in 1894 also in that of human energy needs, in a medical physiology text by Raymond.[7]

Gradually, the biochemistry of pathophysiology and the connection to nutrition was unravelled. In the 20th century, the identification of nutritional components increased strongly. There was the discovery by Casimir Funk in 1912 of 'accessory food factors', essential to the human body, called 'vitamins'. In the first half of the 20th century, these vitamins were gradually further identified, and in the second half, the knowledge about the importance of minerals and trace minerals and other micronutrients increased further.

At present, tables of food composition are publicly available, and it is compulsory for the chemical composition of packaged food products to be provided on the label. Nutritional science as a biochemical science is widely accepted today, and, from a chemical point of view, the design and production of 'healthier' products than the natural ones, e.g. by fortification or enrichment with micronutrients, is a logical step. This chemical way of thinking about nutrition is closely connected to the chemical approach in the agricultural sciences.

However, the development of chemical nutritional science and food production, as part of the general development of materialistic science, industrialisation and urbanisation, was not cherished by all, and thus also raised opposition. At the end of 19th century, in Germany, the 'Reform Movement' emerged, started by Karl Wilhelm Diefenbach. It aimed at a lifestyle that was 'as natural as possible', touching on the various domains of life. For nutrition, the basic principles were to maintain the 'naturalness' of foods to the largest degree possible, and to reflect the perception that, in nature as well as in food products, 'the whole is more than the sum of the parts'. In practice, this meant the consumption of unrefined products and raw foods where possible, the avoidance of chemical additives and a reduced consumption of meat, sugar, alcohol and coffee. The most well-known representatives of this nutritional approach were the physicians Bircher-Benner and Kollath in Europe and Kellogg in the United States.[8]

This Reform Movement also embraced food production principles as they were practiced in organic farming. This farming approach developed in response to the chemical way of soil fertilisation, based on nitrate, phosphorus and potassium, as introduced by Justus von Liebig in 1840. Although Liebig later in life (1861)[9] deviated from his earlier views and pointed to the risks of strong artificial fertilisation to soil depletion and the quality of products, by then his fertilisers were already embedded in general agricultural practice and his later hesitations remained mainly unnoticed.

Yet, some farmers and consumers opposed the narrow chemical approach of artificial fertilisation and developed different farming concepts. An overview of these and connected food concepts is provided by Niggly.[10] Central to these approaches was the aim to maintain and enhance soil fertility and strengthen the natural self-regulating capacity of plants and animals, embedded in an ecosystems approach. This was considered to be the basis for good quality food products. Well-known representatives of one of these practices (the organic movement) were Sir Albert Howard and Lady Eve Balfour, in England, the latter stating 'The health of soil, plant, animal and man is one and indivisible'.[11]

In the 21st century, the two described basic paradigms outlined above still exist side by side. One is the chemical view on food production and food products, characterised by the statement 'food is composed of nutrients' and should be produced as efficiently as possible; the other view considers food products as living entities which 'are more than the sum of the parts' and can be analysed into nutrients, but cannot simply be assembled from nutrients, as if they were Lego pieces. The second approach aims to produce foods with a balance between physiological processes, such as the vegetative and generative process, in order to reach good quality and within the context of a sustainable ecosystem.[12]

Building bridges by modern scientific developments

Recent developments in the modern techniques of metabolomics and other omics may have the potential to build bridges between the two approaches. Omics methods produce a huge amount of data on food products as well as consumers' physiology. These methods have also led to the discovery of a large number of new substances, many of which as yet unidentified. Living products appear to contain many more different substances than were known ever before. In 1999, the number of identified secondary plant metabolites exceeded 100,000 and this number is still increasing.[13] Metabolomics also show the effects of various production methods on different compounds in plants and animals. Apparently, not just single elements can increase or decrease in amount, but within a living system, complete 'clouds' of substances may shift, and this phenomenon allows distinguishing food products according to production system. In this way, these omics techniques are currently being used for identifying products from various production systems. This recently resulted in a chair for 'Food Authenticity' at Wageningen University.[14]

These techniques may bring a subsequent step a little closer, i.e. the ability to perceive the food plant as a whole, as an organism that adapts chemically to different production environments. However, such a step may need a paradigm shift in nutritional science.

Indeed, the enormous amounts of data that are produced by the modern omics techniques pose unforeseen problems. In 2008, Penders published his thesis about his analyses of the large-scale cooperation in nutritional science projects, the Dutch Gut Health Programme and the EC-funded European Nutrigenomics Organisation (NuGO). In his thesis ‘From seeking health to finding healths’,[15] he describes how researchers, in order to make operational modules of a complex problem, tend to modify elements that are part of that problem, according to the research situation. This concerns also the notion of health, which finally results in a diversification of notions and co-existing standards for health, according to the pluriformity of institutional settings. The synthesis of these diverse notions and standards of health is difficult and forms the great challenge of the future, according to Penders.

In the present thesis, we argue that profound knowledge of human physiology, as well as an integrating concept of health, are both essential elements, in order to be able to interpret and to integrate the ‘big data’ which are produced by the modern omics techniques in pluriform institutional settings, in nutritional and physiological research.

Evaluating health effects from foods

In the following chapters, various ways to evaluate the health value of food are described. One way would be analysing the nutritional compounds or nutrients in a certain product and comparing the outcome with the actual state of knowledge on the required daily intake (RDI) of these nutrients. The outcome is described as the ‘nutrient content’ and ‘nutritional value’ of a food product.

More complex is the research on the effects of the consumption of food products. Here, different types of studies are performed. One type is the ‘observational study’, whereby large groups of subjects are studied over relatively long periods of time. As a baseline, a large population of healthy subjects is approached and asked to report, in questionnaires, about lifestyle factors, such as consumption frequency and amounts of different food products. Then, during long-term follow-up, health-related outcomes with respect to these subjects are regularly documented and associated with the lifestyle factors of study. In this way, associations can be found. Such large-scale epidemiological cohort studies have contributed to important basic insights into modern nutritional science. For example, the current, well-known preventive impact of the Mediterranean diet on cardiovascular diseases, is the result of such observational epidemiological studies.

Relationships can be discovered in such studies, and hypotheses about metabolic mechanisms can be formulated. However, causality cannot be proven in this way; for this, a different type of research is needed.

The study design of choice for proving causality of effects is the experimental design. In experimental or so-called ‘intervention studies’, one experimental factor – the food of interest – is the object of study. Moreover, the behaviour of the consuming subjects is under much stricter control than in observational studies. In experimental designs,

confounding can be excluded to a large extent, and, in this way, hypotheses about effects and causal mechanisms can be tested in a rigorous way.
Both humans and animals can be studied in experimental studies, with animals varying from very simple organisms such as fruit flies and round worms (C. elegans), to higher animals such as mice and rats, to the most complex animals such as pigs and monkeys.
In such studies, the effect of a total diet, of single food products, or of separate compounds can be studied.
A complicating factor, even in such strongly controlled research, is that after consumption, physiological processes cannot be fully controlled. Several physiological factors may have an impact on what happens with the consumed food or compound. Is it digested and absorbed by the body, or excreted by the intestines without absorption? Or, once absorbed, does it become physiologically active, or is it excreted by the kidneys without ever becoming effective? And íf active, what is the effect? All these factors may complicate the interpretation of the findings.
For deepening the insight into these questions, besides consumption studies, also laboratory testing of the effects of food compounds on cells or cellular parts can be performed.

Overall, because of this complexity, research on the health effects of nutrition presently consists mainly of research about the effects of diet on diseases, and – in the case of experimental studies – the effects of diet on early indicators of the risk of future disease; for example, blood cholesterol concentrations as a risk indicator for future myocardial infarction. Diseases such as cardiovascular diseases, diabetes and cancer are relatively easier to study than the ‘healthy physiological situation’, in which the organism maintains homeostasis and in which, therefore, disturbances are usually counter-balanced by the organism. Once a disease expresses itself, the homeostatic state of physiology is lost and disturbances appear. These disturbances have been much more and better studied than the ‘healthy situation’ and, thus, the outcome measures for effects are relatively clear. In the first decade of the 21st century, the policy on drawing conclusions about the existence of any health-improvement effects of nutrition, according to publications, remained dependent on whether a clear reduction in risk for a certain disease was scientifically proven. No differentiation was being made between reduction in the risk of disease, the prevention of a disease, the maintenance of health, or the strengthening of health by nutrition.[16,17,18]

This thesis argues for a new approach in nutritional health research, in line with the new concept of health, which addresses the ability to adapt to challenges. Biomarkers need to be identified that can function as signals for physiological capabilities, such as resilience, phenotypic flexibility and the adaptability of an organism.

The following chapters present the first proposals for a new way of research on health in relation to food. In Chapters 6 to 10, an overview of research, by the author and colleagues, on the possible health effects from food products from different production systems is presented. The health effects to which these chapters refer are different from the 'reduction in the risk of disease'. Chapter 8 elaborates on the new concept of health as 'the ability to adapt' (as described in Chapter 3), for evaluating health effects from nutrition. Chapters 9 and 10 present research in which this approach was applied.

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Chapter 6

Organic food and impact on human health: Assessing the status quo and prospects of research

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Abstract

The paper gives an overview of recent studies investigating the health value of organic foods and presents a framework for estimating the scientific impact of these studies. Furthermore,, the problems connected with the different research approaches are being discussed. A number of comparative studies showed lower nitrate contents and less pesticide residues, but usually higher level of vitamin C and phenolic compounds in organic plant products, as well as higher levels of omega-3 fatty acids and conjugated linoleic acid in milk from organically raised animals. However, the variation in outcomes of comparative studies is very high, depending on plant fertilis, ripening stage and plant age at harvest, and weather conditions. Moreover, there appeared no simple relationship between nutritional value and health effects. It is difficult therefore to draw conclusions from analytical data about the health effects of organic foods. Some *in vitro* studies comparing health-related properties of organic vs conventional foods showed higher antioxidative and antimutagenic activity as well as better inhibition of cancer cells proliferation on organically produced food. If 'health effects' are defined as effects on defined diseases in humans, evidence for such effects is presently lacking. Animal studies carried out so far have demonstrated positive effects of organic diet on weight, growth, fertility indeces and immune system. Recent human epidemiological studies associated consumption of organic foods with lower risk of allergies, whereas findings of human intervention studies were still ambiguous. The hypothesis might be that organic food increases the capacity of living organisms towards resilience. To confirm this, effect studies on specific markers for health are necessary.

Key words: review; organic food; health; humans; intervention; observational; *in vitro* studies

Introduction

Consumer studies continue to show that expectations concerning health effects of organic food are about the strongest motives for consumers to buy organic products and research results on this topic can count on high societal interest.[1-3] However, until now these expectations lack sound scientific proof.[4] Different kinds of research are being performed to investigate the health value of organic products, compared to conventionally produced products. An increasing number of studies are being published, including studies comparing the contents of ingredients of products from conventional and organic production systems, as well as review studies. Apart from this, a much smaller number have been published on effects of organic food consumption. These include animals and human studies on bioavailability and health effects, *in vitro* studies comparing effects of organic and conventional products on different parameters in the laboratory. In this paper an overview of recent studies on the topic is given, with a framework for estimating the scientific value of these studies. In addition, the problems connected with the different approaches are being discussed. A hypothesis is presented about the possible health effects that organic products might have, and suggestions are made for future research.

Comparative studies on nutritional value

Plant products

A number of studies have looked at the content of primary and secondary metabolites of foods from different production systems, e.g., organic and conventional systems. The older studies have been reviewed.[5,6] The main conclusion was that organic products had a higher dry matter and lower nitrate content and contained less pesticide residues. Regarding vitamins they concluded that there were trends towards higher vitamin C content in organic products, while data on mineral content were inconclusive. Since then over 200 papers concerning nutritient content of organic vs conventionally produced foods have been published and it is evident that the interest in this field increased dramatically over the years. However, conclusions since 1997 have not changed as dramatically. In plants, the focus during the last 10 years has been on the contents of vitamin C, carotenoids and phenolic compounds. Various fruits and vegetables have been investigated under different climatic conditions, with different varieties and on different soil types.

In a review paper, Worthington[6] presented a meta-analysis showing that in most studies the level of vitamin C was significantly higher in organically than in conventionally produced plant foods ones. Also in more recent studies, higher vitamin C contents were found in many organic products, e.g., peaches[7] and tomatoes[8,9], although other studies reported similar or lower contents of vitamin C in organic tomatoes[10], broccoli[11], bell peppers[9], pear and peach.[7] A higher carotenoid content was found in organically grown sweet peppers, yellow plums, tomatoes and carrots[9,12,13] whereas others[4,15] found lower or similar contents of carotenoids in organically grown blanched carrots and tomatoes. From a study of Barrett *et al.*[10] it is known that the content of carotenoids may depend on soil type, genotype, as well as the fertilisers and pesticides used. This might explain the inconsistency of the findings in the above-mentioned studies.[10]

An increasing number of studies have measured the content of phenolic compounds that might have a chemopreventive role in humans by modulating the cancer cell cycle, inhibiting proliferation and inducing apoptosis. A number of studies have actually shown that the content of phenolic compounds is higher in organic products[7-9,12,16-18], whereas other studies[9,12,19] have found similar or lower content of phenolic compounds in organic products

In most studies comparing conventionally with organically grown cereals, higher levels of proteins and amino acids were found in the conventionally produced grain (reviews by Heaton[20], Worthington[6] and Benbrook *et al.*[21] and in recent studies.[22] The higher N-fertilisation in conventional production system is very likely to explain this difference. Some studies also observed that the quality of the amino acids was higher in the organic products than in the conventional products, meaning that more essential amino acids were available in the organic grains.[22] These latter findings were not confirmed in other studies.[23] Apart from the described potentially beneficial components, conclusions can be drawn concerning lower amounts of pesticide residues[24,25], nitrates[26,27] and equal or lower amounts of mycotoxins[25,28] in organic plant crops.

Animal products

Also in animal products differences between organic and conventional production systems have been observed. Milk studies from the Netherlands, UK, Denmark and the USA have shown that milk from organically raised animals has higher contents of n-3 linolenic acids and conjugated linoleic acid (CLA) comparing with milk from conventional systems.[29,30] Such differences with conventionally raised animals are observed especially in summertime, when the organically raised animals have their outdoor grazing facilities. A recent study from the UK showed that milk from low-input systems, both organic and non-organic, has higher contents of n-3 linolenic acid and CLA, although highest contents were found in the non-organic low-input system. Outdoor grazing, a high biodiversity in pastures, low levels of concentrates and no silage feeding were found to be predominant factors for beneficial milk fatty acids composition.[31]

Most recent are two review papers from the French and the British Food Standard Agencies, both of which published in the summer of 2009, but presenting quite different results.[32,33] The French AFSSA paper[32] mentions the earlier described results of a higher dry matter content, more minerals (Fe, Mg) and more anti-oxidants like phenols and salicylic acid in organic plant products, as well as more polyunsaturated fatty acids in organic animal products, apart from 50% less nitrate, 94-100% of products without pesticide residues and equal amounts of mycotoxins. The British FSA paper[33] describes a systematic review of 50 years of publications, with strict inclusion criteria, and mentions more phosphorus and acidity and fewer nitrates in organic products, but no other differences. However, the review did not consider most of the studies presenting data of well-controlled field trials. Contaminants like mycotoxins, pesticide residues and heavy metals were not included in the review paper. The latter paper has given rise to a fierce debate concerning the in- and exclusion criteria, which is still ongoing at the moment the present paper was submitted.

Translation of compositional information to impact on human health

Comparative studies on chemical composition of food products from organic and conventional production systems are valuable and may provide indications for possible health effects. However, it should be recognized that hypotheses about effects of compounds are often revised. Considering that plant physiologists estimate the plant world to contain up to 75,000 or even 100,000 different compounds, being 7,500 to 10,000 per plant, that act synergistically in the plant organism, it becomes clear that even advanced methods, like in systems biology that analyse hundreds or even thousands of compounds, only portray the top of the iceberg of plant chemistry. Let alone the interaction between such a complex food product and the likewise complex organism of the consumer. The first clear complicating factor is the way in which compounds are resorbed by an organism, measured as bioavailability. Secondly, it is not predictable how the consuming organism will react biologically on a food product, as this depends on individual constitutional differences, as well as the actual health status. And in real life, products are integrated in a food matrix, with chemical interactions between products. This complicates the question about hypothesised effects even more. So some reticence in speculations about effects based on analytical outcomes is due here. This is why studies that measure factual effects of food products are more informative, although not simple. Some approaches will be described, with a framework for estimating the scientific value of these study designs.

Types of effect studies for organic products

Intervention studies

Societal interest in health effects of organic products comes from consumers. Seeking for scientific proof to answer the inquiries of this group, studies in humans are most convincing, especially so-called ‘intervention studies’. In this study design as many factors as possible need to be controlled for a group of people (as so many factors other than nutrition do affect people’s health and well-being) and only the food under study is clearly varied in order to let possible effects become visible. So either a set-up needs to be created where a group of people is brought voluntarily into a controlled situation, or special situations need to be found where groups of people live daily under the same conditions and in the same routine, like children’s homes, monasteries or prisons. In such a controlled situation ideally two matched groups should consume parallel either organically or conventionally grown food, blinded. Or a ‘cross-over’ situation is created where the different test foods are presented, one after the other with sufficient time in between. Health effects should be measured using ‘biomarkers’, identified as reliable reflection measurements for a person’s health status, and that can in such a study design be measured in all study objects at the same moment. The choice of food products and the way in which they are presented are factors to take into consideration. This point will be touched upon later.

Observational studies

Another way to study health effects in humans are so-called ‘observational’ or ‘epidemiological’ studies, where a large group of people is studied using questionnaires usually supplemented with some measurements in a smaller part of the group. Control is much less as people themselves report. Investigations can look back at eating habits in the past, being ‘retrospective’, or follow a group from a certain moment into the future, being ‘prospective’. Questions need to address many more factors than food, e.g., life-style factors and social status, to be able to rule out confounding. So a large group of people needs to be included in the study.

Intervention studies in animals

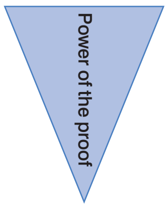
As highly controlled blinded human dietary intervention studies, especially if intended to examine long-term physiological responses, are very expensive and difficult to realize, health effects of foods are usually tested in animal models. Similar to human intervention studies, in such experiments laboratory animals such as rats, mice, chickens, rabbits are fed organically or conventionally grown feeds, and selected physiological parameters reflecting measurements of health status are analysed. By choosing genetically homogenous populations of animals and keeping them under highly controlled conditions it is easier to point out health effects of a diet. Moreover, the short life cycle of animals allows examining effects of diet on more than one generation. Systematic reviews of such animal studies can give indications of possible health effects, though differences between animals and men need to be taken into account. Final confirmations of hypothesised effects need eventually to be verified in humans.

In vitro studies

The so-called *in vivo* studies, referring to experimentation using a whole, living organism, are often substituted/preceded by low-cost *in vitro* experiments. This type of research aims at describing the effects of experimental variables on organism's constituent parts (e.g., organs, tissue- or cell cultures, cellular components) in a controlled environment outside the organism (test tubes, Petri dishes). *In vitro* studies are highly focused, enabling to deduce mechanisms of actions and to control for many confounding variables. However, weakness of this type of studies is the uncertainty that the effects observed at cell level would occur in the 'real world' of the complex living organism.
The scientific value of different study designs concerning the comparison of organically and conventionally produced food is presented in Table 1.

Table 1 Scientific value of different study designs for comparing organically and conventionally produced food, with examples

Study design	Examples
Intervention studies	Controlled studies in humans
Observational or epidemiological studies	Prospective cohort studies
	Retrospective cohort studies
Intervention studies	Controlled studies in animals
Supportive studies	Bioavailability studies
	In vitro studies



Adapted from GRADE Working Group[60]

Recent *in vitro* studies

To our knowledge, in recent years, two *in vitro* studies have been published comparing health-related properties of organic vs conventional foods. The first study analysed antioxidative and antimutagenic activity of organic vs conventional green vegetables (qing-gen-cai, Chinese Cabbage, spinach, Welsh onion and green pepper).[34] The authors found antioxidative activity in the organic vegetables to be much higher than that in the conventional ones. Moreover, organic vegetable juices exhibited significantly stronger suppressive effects against mutagens. The second study compared the effects of extracts from organically and conventionally grown strawberries on the proliferation of colon- and breast-cancer cells [35]. The results demonstrated higher antiproliferative activity of extracts from organically grown strawberries on both types of cancer cells, which was probably due to a higher content of secondary metabolites with anticarcinogenic properties in these fruits. The results suggest a possible mechanism by which organic foods could reduce human cancer risks.

Recent animal studies

During the last 50 years several animal dietary intervention studies have been carried out investigating the health effects of organic vs conventional foods.[36] Most of these studies confirmed beneficial effects of organic feeds on development rate and reproductive abilities of laboratory animal.[37-39] Moreover, animal studies published in recent years indicated increased immune parameters in organically fed laboratory animals. In a dietary study with rats, comparing the effects of protein-poor organic and conventional feed Finamore *et al.*[40] found higher levels of stimulated lymphocyte proliferation in the rats fed organic feed. Lauridsen *et al.*[41] found higher immune system reactivity of organically fed rats, indicated by the level of IgG in blood serum, as well as a lower amount of fat tissue and more relaxed behaviour. A pilot experiment of Barańska *et al.*[42] showed higher splenocyte proliferation in male organically fed rats. According to a study performed in the Netherlands[43], chickens fed an organic diet had lower body weight, higher immune reactivity and stronger catch-up growth after a challenge. In this study the concept of 'resilience' was proposed, as to indicate physiological elasticity to come back to homeostasis after a disturbance. Resilience is a well-known concept in ecology as well as in psychology[44], and is worth investigating for its value in evaluating physiological effects of organic food products, as these are grown with the aim to be more 'robust' than conventional products.

In summary, animal studies on the health effects of organic vs conventional feeds are sparse. Therefore further, well-planned long-term experiments are necessary to evaluate overall health status of laboratory animals fed on feeds from different agricultural production systems.

Recent studies in humans

Observational studies

To our knowledge, only a few observational studies investigating the health effects on humans of organic compared with conventional foods have been performed in the recent years. According to one of these studies, commonly named the PARSIFAL study (14,000 children, 5 European countries), children representing an antrophosophical lifestyle (including biodynamic and organic food) were found to have less allergies and a (not statistically significant) lower body weight compared with a group consuming conventionally produced foods.[45] At the same time the results of the KOALA Birth Cohort Study in the Netherlands (about 2,700 newborns) associated the lower eczema risk in children at the age of 2 years with the consumption of organic dairy products.[46] Moreover, organic dairy consumption resulted in higher CLA levels in breast milk of their mothers.[47] According to a study of Rembiałkowska *et al.*[48] consumers of organic foods assessed their health state significantly better than consumers of non-organic food. However, apart from the organic diet, this might also be related to several aspects of consumers' lifestyle (e.g., nutritional pattern, living conditions, physical activity, ways to manage stress).

As was mentioned above, pesticide residues form part of the dangerous food contaminants known to exert genotoxic, carcinogenic, neuro-destructive, endocrine and allergenic effects, and are usually found in higher contents in conventionally produced plant products. There is scientific evidence that dietary exposure of children to organophosphorus pesticides, measured as the level of pesticide metabolites in urine, is much lower on an organic compared than on a conventional diet.[49] It can be concluded that consumption of organic foods provides protection against exposure to organophosphorus pesticides commonly used in agricultural practices.[50]

6

Intervention studies

As several others have stated previously, interpretation of the results from comparing organic and conventional foods are extremely difficult due to differences in methodologies related to the use of different varieties, growing conditions and sampling procedures. Furthermore, the content of nutrients and secondary metabolites in the plants cannot be directly correlated to a potential health effect. First of all, the contents of primary and secondary metabolites in the food do not give any indications of how much they are actually absorbed, as the absorption depends on a number of factors, such as the amount of promoters and inhibitors available in the food, as well as the food matrix itself. In order to obtain more information on uptake of valuable compounds, studies on bioavailability and effects on specific markers for health are necessary.

To our knowledge only 6 dietary controlled human intervention studies comparing organic and conventional foods have been performed. Two of these are small single-meal studies comparing the effects of organic and conventional apples or red wine consumption respectively.[19,51] In both studies the postprandial effect on biomarkers for redox-processes was measured. Neither study found any difference in redox markers between the organic and conventional products.

In two other studies, volunteers were given either organically or conventionally produced carrots or tomato purée in addition to an otherwise habitual diet for 2-3 weeks.[8,15] In the first study[15] no effect of the particular diets on basic haematological parameters, vitamin C and E in plasma, or LDL oxidation was observed. Carrot consumption had also no effect on the antioxidant status of plasma. However, plasma lutein increased significantly in the group consuming organic carrots. In the second study, in which volunteers were fed organically or conventionally produced tomato purée for three weeks in a parallel design, no differences between bioavailability of lycopene, β-carotene or vitamin C between organic and conventional tomato purée were observed [8]. The reason for the lack of differences between groups consuming organic and conventional carrots and tomato purée could be that the products tested were given in addition to a habitual diet, which could have diluted any effect that there might have been between the production methods. In order to assure that such dilution does not appear, fully controlled dietary studies are needed. Only two such studies have been done so far.[52,53]

A small and very poorly described Italian study intended to compare the effects of an organic vs a conventional Mediterranean diet given to 10 healthy men for 2 weeks. According to the results, the plasma antioxidant status following the organic diet appeared to be higher than following the conventional diet. As no standard deviations were given it is not possible to conclude whether or not the difference was statistically significant. Furthermore, it looked as if the study was not randomised, which means that the observed effect might be due to later harvesting so that more mature products were used in the second period of the study. In the same study, antioxidant activity was measured in a number of fruits and vegetables, and in wine and milk. In the majority of these products the activity was highest in the organic products.[52]

The other study was a fully controlled dietary intervention with organic or conventional diets fed to 16 male and female volunteers in a randomised cross over design for 2 x 3 weeks.[53] The study aimed at a comparison of the intake and excretion of selected flavonoids, and the plasma levels of known oxidative defence markers in both groups of volunteers. The organic diet resulted in higher urinary excretion of quercetin and kaempherol, while no difference was observed between diets in respect of the excretion of other analysed flavonoids. Most markers of antioxidative defence did not differ between the diets. However, intake of an organic diet resulted in an increased protein oxidation and a decreased total plasma antioxidant capacity compared to baseline. In this study the vegetables were collected by one distributor from established organic and conventional producers within similar geographical locations. However, for some of the products the producers used different crop varieties so that it cannot be concluded whether the observed differences in the human intervention study were due to the differences in varieties as part of the production method or to differences in production method.

Discussion

The overall number of studies comparing nutritional value of organic vs conventional foods is growing. There also is an increasing interest in investigating the health effects of organic food consumption. Results of comparative studies, as well as *in vitro* analyses, animal intervention trials and human observations are promising. However, the results are still insufficient to formulate explicit conclusions.

One problem is the variation in outcomes of comparative studies, which is very high depending mainly on crop fertilisation, ripening stage and plant age of the plant at harvest, and weather conditions. First, the amount of fertiliser used has a large impact, in conventional as well as in organic production.[54] Second, also the type of fertiliser is of influence, being either quickly available nitrate in inorganic fertiliser, or slowly available nitrate in organic fertiliser. Generally, large amounts of fertiliser enhance vegetative growth and the connected formation of primary nutrients, like proteins and carbohydrates, while the generative growth of these plants and connected formation of secondary metabolites, like polyphenols and vitamins, can become inhibited.[55] The ripening stage and the age of a plant at harvest also influence the amount of desired compounds. As the generative stage follows naturally the vegetative stage, a harvest at a too early stage might result in sturdy well transportable products that at the same time have a low contents that are desirable (health promoting, and bringing colour, taste and smell). It is questionable if artificial ripening through ethylene brings about the same quality of ripening as when the ripening takes place on the plant under influence of the sun. Weather is another important factor strongly influencing composition of plant products. Observed year-to-year variation due to weather conditions is often larger than the differences between cultivation systems.[55-57]

The lack of a straightforward relationship between nutritional value and health is another reason why it has been difficult so far to draw conclusions from comparative studies on the health effects of organic foods. As the bioavailability of chemicals is limited and can be affected by numerous factors, the contents of nutrients and secondary metabolites in plants cannot give straightforward indications of their health effect.

When intervention studies on health effects are performed several choices concerning the consumed food products need to be made. Least preferable are random market samples, as no indication about production conditions is available.[58] Products from controlled trials have the advantage of the control. However, they lack the embeddedness in a complete farming system, which for organic products might be a disadvantage. Another possibility is the use of products from 'best-practice farm-pairs', a conventional and neighbouring organic farm. Choice for the same or acceptance of different varieties is also a point of discussion. It can be argued that the same crop varieties (or animal breeds) should be used in order to avoid an important factor of variability, as it is known that different crop varieties can contain quite different contents of the same nutritive substances. It can, however also be argued that organically managed soils are so different that adapted varieties are needed with different root systems. That implies that each production method should use its own varieties. A last choice is if analytical differences in feeds are accepted as being typical characteristics of these feeds inherent to the production system where they originate from, or that it is necessary to compensate for those differences in order to allow research to identify (possible) differences other than those at macro nutrient content level. A factor of discussion in health effect studies is the choice of health outcomes that are considered relevant for conclusions. Recently the systematic review of the FSA[59] took 'health outcomes' as effects on defined diseases in humans and concluded that evidence for health effects is lacking. It is questionable if foods from different production systems will have such 'strong' effects of influencing existing pathologies, while yet possibly still support health. On the basis of the experiments done so far a hypothesis might be: 'organic food consumption may increase the capacity of living organism towards resilience'. However, to confirm this statement it is necessary to perform more effect studies on specific health markers.

Towards the future

With the knowledge gathered in the studies thus far, indications have been found of potential health effect of organic food for humans. To further elucidate this relationship, future studies can be performed in several areas.

Comparative studies on nutritional value

Compositional data from studies comparing organically with. conventionally produced products are important mainly to obtain more insight into the relation between cultivation practices and nutritional content. This will enable the production of best quality products. As already mentioned above, the relationship between the nutritional value of a product and health is difficult to predict and we therefore suggest putting the focus of future research more on studies in animals and humans. For such studies it will be important to define markers, e.g., fingerprints or other for representative organic food products.

Intervention studies

To study the effect of a specific food or a diet on health, intervention studies can be done in animals as well as in humans. For such studies only best quality products from the production systems are to be used to ensure good research on the potential impact of the organic food. The hypothesis of the possible increase of the capacity of resilience as a result of organic food consumption should be studied using challenges. For the studies in humans, it is important to define specific biomarkers for expected effects from representative food products.

Observational studies

Big population studies in humans are important to confirm health effects within a large population. Such studies might show unexpected relationships that cannot be investigated with intervention studies because of the time frame and logistics. As long as biomarkers do not give clear answers and the lag time before observable health effects occur is too long, observational studies can fill this gap. It is efficient to attach the organic question to big, already ongoing studies.

In vitro models

Development of *in vitro* models could be valuable to elaborate mechanisms by which organically produced foods might influence the health status.

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Chapter 7

Feeding trials in organic food quality and health research

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Abstract

Feeding experiments comparing organically and conventionally produced food are performed to assess the overall impact on the animals´ health as a model for the effects experienced by the human consumers. These experiments are based on systems research and characterised by their focus on production methods, whole food testing and procedures in accordance with the terms of organic farming.

A short review of such experiments shows that the majority of these tests revealed effects of the organically produced feed on health parameters such as reproductive performance and immune responses. Systems research is not just about simple cause-effect chains, but rather about the pluralism of interactions in biological networks; therefore the interpretation of the outcome of whole food experiments is difficult. Furthermore, the test diets of organic and conventional origin can be constituted in different ways, compensating for or maintaining existing differences in nutrient and energy contents.

The science-based results suggest positive influences from organic feeds, but there is still a need for confirmation in animals and, finally, in humans. For this purpose animal feeding trials with feed from different production systems should be conducted, with the aims to define health indicators and to establish biomarkers as a basis for future dietary intervention studies in humans.

Key words: feeding trials; organic feed; health effects; biomarkers

Introduction

Organic agriculture as defined in the EU regulation (EC) No 834/2007 does not use synthetic fertilisers and pesticides, but it is not 'just without' these substances. Organic agriculture optimises production, through modern farming management skills, along with maintaining the inborn capacity of plants and animals to be healthy without addition of synthetic pesticides and antibiotics. The aim is to create an ecologically balanced system on the farm at its specific location. Organic farm management does not follow one set of rules, but is an individualised skilled application of general principles. It has previously been shown that organic agriculture is advantageous for soil fertility,[1] for biodiversity,[2] for sustainability[3,4] and for animal welfare and health.[5] The vision as stated by Lady Balfour,[6] the founder of the Soil Association in 1946 – 'Healthy soil, healthy plants, healthy people' – can still be regarded as the key note of organic farming.

Consumers expect organic foods to be healthier than conventionally produced foods,[7] but so far, research has not been able to prove this. The International Research Association for Organic Food Quality and Health, (FQH), aims to investigate and develop a scientific conceptual basis for organic food quality, as well as novel methods to examine food quality and to study the influence of organic food on human health.[8] The association consists of research institutes that connect in their activities to these objectives and researchers of these institutes work together to attain these aims.

The aim of this paper - by authors who are all representatives of member institutes of FQH – is to describe the state of the art of animal experiments used for comparing the impact on health and preference of organically and conventionally produced food. Furthermore, necessary criteria for animal feeding experiments that do justice to the concepts of organic agriculture are clarified.

Little is known about possible physiological effects of organic food in humans due, in part, to the lack of clear health biomarkers. Therefore, animal feeding experiments are warranted, aiming at identification of biomarkers suitable for human intervention studies. The animal studies described in this paper have focused on the possible differences between the consumption of products from modern conventional and certified organic production and from organic or mineral fertiliser regimes. This paper is focused on scientific studies, mainly performed on laboratory animals, not on differences in the health status of farm animals in organic and conventional production systems.

The described animal experiments have the purpose of using animals as models for humans. In the field of nutrition in general, experimental animals are either used to assess the risk of toxic food substances, or to prove beneficial effects of certain food additives and components, or more generally to study physiological aspects such as absorption, metabolism, and function of nutrients. The findings from animal studies can be used as guidelines for humans, although the extrapolation of animal results to humans has to be done with great care.

Epidemiological studies have repeatedly shown that high intake of fruits and vegetables decrease the risk of lifestyle-related diseases and increase longevity.[9-13] These beneficial effects are attributed to bioactive phytochemicals as well as vitamins, trace elements and fibres.

In both cases, testing negative effects of toxic substances or positive effects of health-promoting nutrients, the focus is on the investigation of single cause-effect chains. But in the context of organic agriculture and food quality research, including feeding experiments, the epistemological background is based on the holistic perception of systems. Thus a whole food product is not reduced to a set of chemicals, but is seen as a dynamic, hierarchically organised unit in which short linear cause-effect chains are modulated by synergistic, additive and reductive interactions. Foods can have effects that are not traceable to the components, but emerge from the interaction of these components. This notion is well expressed by the dictum: 'The whole is more than the sum of the parts'. Differences between the effect of single compounds and whole products have been shown in several intervention studies.[14-19] Living organisms, concerning agricultural foods in the context of nutrition, (self-) organise the contained compounds ideally into a homeostatic status.[20] Organic agricultural research is based on the hypothesis that the coherence of this organisational structure is influenced by production measures. This production-dependent intrinsic property or 'inner order' might have an impact on health and account for feeding trial outcomes irreducible to the chemical composition of the test diets. The consequence for the animal feeding design connected to organic agriculture is the use of whole foods, not purified diets.

The focus of this paper is on plant-derived feeds, since, so far, mainly organically produced crops have been investigated in feeding trials.

Feeding experiments in organic food quality research

From the described holistic perspective in organic agriculture, research concerning food quality and connected health effects should not exclusively rely on the composition data of the food, but also characterise the food products by a more comprehensive view of food quality. This could be based on systemic properties like integrity, inner order and 'inner quality', referring to aspects of an organisational structure which cannot be explained solely out of its components. Several holistic methods aiming to measure aspects of these quality aspects are currently in a process of standardisation and validation (e.g., low-level luminescence and bio-crystallisations).[21, 22] Likewise, feeding experiments in organic quality research are performed to assess the overall effect of differently grown feeds on the animals´ health. These experiments are characterised by the following features:

- focus on production method;
- whole food testing;
- procedures in accordance with the terms of organic farming.

Focus on production methods

The aim of the comparative feeding experiments described herein is to reveal food effects, e.g. on general health, eating behaviour, breeding performance and immune reactions, that can be traced back to production methods. This represents a new approach currently only used in organic quality research.

The flexibility within the regulatory frame work is lower in organic as compared with conventional agriculture, where the range of quality produced stretches from almost organic, but not certified, to industrial, with the highest external inputs at present. Therefore, in such comparative studies the way the different foodstuffs have been produced has to be described in detail. In some studies factorial field trials are used to control all growing conditions and to emphasise the potential impact of defined applications: for instance, fertilisation and/or plant protection. The advantage of comparing products from operating farms in the 'farm-pair approach' is the simultaneous inclusion of many additional factors such as crop rotation, intercropping, soil quality depending on long-standing treatments and management skills. Although this approach is very complex, it pays tribute to the notion that organic farming is an individualised application of general principles. In this case the conventional products have to originate from neighbouring farms with the same soil and climatic conditions and should reflect region-typical farming methods. Both approaches are important contributions to quality assessments: feeding tests based on factorial field trials are important for basic research, whereas farm-pair comparisons reflect realistic conditions and are, therefore, of more interest for the consumer.

So far, the scientific approach has been to compare the same diet composition of the same cultivars. But one important feature in organic farming is the recognition of the necessity to use varieties adapted to the specific organic conditions of fertilising,[23] next to the aims to reintroduce old varieties to conserve genetic resources and to offer more taste diversity. Therefore, in a systems-oriented feeding design, the use of different cultivars as typical of the respective cultivation system can also be introduced.

Whole food testing

The epistemological background of whole food trials postulates - as mentioned before - that single components consumed as integrated part of the food could act differently as compared to results obtained from testing the isolated compound.

Concerning the test feed preparation, two approaches are used: either all or the main nutrient differences are levelled by adding the lacking components to both diets, or existing compositional differences are preserved and seen as intrinsic quality differences, thus being part of the study design. The latter approach involves the entire food system by accepting compositional differences as cultivation-inherent properties reflected in different feed effects. It should be taken into account that deficiencies should be avoided as these can confound results. Since the analytical differences of main nutrients between conventional and organic products are generally low, both variants can be expected to fulfil the metabolic needs of the test animals.

The influence of the feed-processing steps on cultivation-dependent quality parameters must be taken into account if cultivation is the main focus. Intensive processing such as the use of heat, pressure and freeze drying might denature structures, thus possibly obliterating primary production-dependent differences.

However, processing steps may have beneficial effects on digestibility and availability of health promoting compounds (e.g., lycopene in tomatoes)[24] could be tested in a dietary intervention design where the primary focus is on processing. Feeding trials with differently processed food, either conventional or organically, are still rare in the field of organic quality research,[25] but there is increasing demand for such studies to define and optimise organic processing methods. Pesticide residues should be under the safety limits to reflect realistic conditions. Questions about harmful effects from pesticides require separate focused research designs.

The interpretation of results from feeding trials, where the test diets were comparable in nutrient contents, poses a challenge which needs special attention.

Williams[26] remarks that the very small differences in nutrient contents of crops grown under the two systems (organic and conventional) would be very unlikely to provide a nutritional basis for the differences in reproductive performance or immune reaction in these animals. Therefore, new hypotheses regarding systemic topics are needed, which could result from the development of holistic quality parameters and be measured in a descriptive way or in phenomenological observations. But phenomenological observations, although playing a valid role in science, do not replace the need for understanding and relating the findings to underlying principles. Biological systems are complex systems, still barely understood, especially concerning the influence of nutrients on gene expression affecting metabolic pathways.

The current approach of systems biology - analysing small segments and developing data integration models to elucidate complex interaction networks - relies on the notion that well-designed experiments will eventually lead to an understanding of them all. Possibly, several key functions can be isolated, valued separately and then used for a joint single score upon which the quality assessment can be based.[27] On the other hand, results from holistic quality assessment methods based on a property, reflecting the overall performance, could provide more meaningful answers than analytical trait scoring.

Procedures in accordance with the terms of organic farming

Apart from the systemic approach, 'organic' feeding trials should be conducted in agreement with the principle of ethics implemented in the organic movement (IFOAM principles: health, fairness, ecology and care; http://www.ifoam.org/about_ifoam/principles/index.html). These principles include the avoidance of painful procedures, the opportunity for the animals to have social contacts and the use of adequate feed according to the test animals´ nutritional needs. Thus beneficial and negative health effects should be linked to quality differences alone and not to stress and/or nutritional inadequacy.

Variables and parameters investigated in health research on organic food

The variables investigated in comparative feeding studies comprise feeding behaviour and consumption, fertility parameters, as well as biomarkers of health, e.g. weight gain and growth, blood parameters, immune status, organ function and post-mortem analyses.
Feeding experiments with laboratory animals are routinely used in toxicology and nutritional research, but in the context of investigating potential health effects of organic food the approach has focused on synergistic and additive interactions of whole foods (Table 1).

Table 1 Different approaches of feeding trials in nutritional research

Variables	Approach 1	Approach 2
Type of trial	Animal experiment (subject of authorisation)	Animal experiment (subject of registration)
Test object	Single compound	Whole food
Type of effect	Linear (dose dependent)	Synergistic (network dependent)
Test aim	Health risk or benefit of test substance	Health benefits of whole food

In the following, feeding trials with different research questions are summarised. The studies used parameters aimed at detecting potentially different effects of organic and conventional production methods, as well as differences in fertilisation techniques. Studies from before implementation of the EU-regulations on organic agriculture are included, as they may contribute to indications about where to look for physiological effects.

The influence of different fertilising and processing methods

The first feeding trials focusing on production methods were conducted in the 1930s. Their purpose was to compare the effects of. biological-dynamical vs. mineral fertilisers on product quality and animal health. At that time only biodynamic farming had been regulated (1928). Wöse *et al.*[28] give a very comprehensive description of these early endeavours (Table 2).

The majority of the studies showed, that animals fared better with biodynamic (compost, manure), as compared to minerally fertilised feed,[29-34] while a few observed no effect.[35,36,37]

Neudecker[37] found no differences concerning fertility parameters between the feeding groups. In this case the test products were carrots and potatoes, which were boiled, freeze-dried and pressed into pellets. It could be questioned whether this intensive processing - the use of heat, freezing and pressure - might change primary

Table 2 Overview feeding experiments with different fertiliser applications (adapted from Wöse *et al.*[28])

Author	Objective	Feed	Animal
McCarrison (1926)	Cow dung vs mineral fertiliser (NPK)	Wheat	Rats
Pfeiffer (1931)	Biodynamic vs. mineral fertiliser	Wheat	Mice
Pfeiffer and Sabarth (1932)	Biodynamic vs. mineral fertiliser	Wheat	Chicken
Scheunert (1935)	Unfertilised vs. mineral fertiliser	Cerials, vegetables, milk, beef	Rats
Miller & Derma (1958)	Unfertilised vs. dung vs. mineral fertiliser	Wheat	Rats
Aehnelt & Hahn (1965)	Cow dung vs. mineral fertiliser	Hay	Bulls
Aehnelt & Hahn (1973)	Biodynamic vs. mineral fertiliser	Hay, carrots, kohlrabi	Rabbits
Bram (1974)	Biodynamic vs. mineral fertiliser vs. liquid manure	Hay, kale, carrots, kohlrabi	Rabbits
Alter 1978	Biodynamic vs. mineral fertiliser vs. liquid manure	Pasture, hay, kale, kohlrabi, carrots	Rabbits
Neudecker (1987)	Organic vs. mineral fertiliser	Carrots and boiled potatoes were freeze-dried and fed as pellets	Mice, rats

quality differences in the products. Little research has been done in this area but its worth more focused research. On the other hand, in a still running feeding experiment with rats it has been found that the level of secondary plant compounds (polyphenols and carotenoids, in 4 replicates) was significantly higher in the processed rat feed obtained from the organic crops compared to rat feed based on the conventional crops. This means that higher nutritive value of the fresh crops obtained from the organic cultivation had not been changed during the processing procedure, which observed temperatures below 50° C.[38]

The influence of farming systems

Gottschewski´s[39] feeding experiment with rabbits was among the first investigating food from different farming systems as opposed to different fertilising methods. He used feed which was produced according to the biodynamic regulations, established

Assessed parameter	Results
Growth rate	Rats fed with wheat grown with cow dung showed a better growth rate after 72 days (22,5% more weight)
Pup survival rate	Until the 9th week about 50% more pups survived when fed with biodynamic fertilised wheat (biodynamic, 8.6%; mineral,16.9%)
Egg production	7 months: org.121.3 vs. min. 97.7 eggs 9 months: org. 192 vs. Min. 150 eggs hatching: org. 68% vs. min. 35% eggs with chicks
Reproduction	Mineral: larger litters Unfertilized: better survival rate
Growth rate	No difference
Fertility	Cow dung: better semen motility
Fertility organ-centred	All fertility parameters better with biodynamic (ovulation points, fertilised eggs, weight of ovaries)
Cell count and nucleoli size in adrenal cortex	Group fed with minerally fertilised products showed a reduction of cells and nucleoli size
Fertility parameters of males and females	No difference
Fertility parameters of males and females	No difference

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in 1928 as certified Demeter quality. His findings were later corroborated by two more rabbit experiments, observing significantly better rearing successes due to fewer perinatally dead and more weaned pubs in combination with a superior weight development in the organically fed rabbits.[40,41] The feed in the two latter projects was produced according to the national regulation for organic agriculture in Austria, established in 1983.
Very similar results could be obtained when feeding laboratory rats with organic *vs.* conventional feed.[42] Again, in the organic group significantly fewer offspring were stillborn, or died within the first week of their lives; the survival rate until weaning time at the age of 28 days and the weight development were slightly higher, and also the weight gain of the female rats in connection with litter size and pup weight during lactation was significantly higher. In this case the diets were supplemented up to the same nutritive quality. Any primary differences were adjusted by the addition of trace elements, minerals and vitamins. The basic diet was pelleted, but next to this fresh carrots and common beets were apportioned daily. This suggests that measuring the main nutrient concentrations does not suffice to predict health effects.
Table 3 provides an overview of feeding experiments with a systemic approach.

In search for health biomarkers

In a recent study of three generations of rats, the objective was to define which measurable aspects of health (if any) would be most affected by differences in production methods, as information for more targeted future studies (Table 4).[43] Three iso-energetic and iso-nitrogenous diets composed of vegetables and a high content of rapeseed oil (13 %) produced according to each of three different cultivation systems were used in the study. The differences between the three diets were three combinations of cultivation strategies used to grow the ingredients: ‘Organic’ - low input of fertiliser through animal manure and without pesticides; ‘Minimally fertilised’ - low input of fertiliser primarily through animal manure and with pesticides; ‘Conventional’ - high input of mineral fertiliser and with pesticides.
Even though the dietary treatments were supplemented until similar in terms of nutritive quality, some notable differences appeared with regard to some of the measured health biomarkers in the rats that have until now not been assayed in similar comparative studies. Among these health-related biomarkers were concentrations of α-tocopherol and immunoglobulin G, daytime activity, volume of adipose tissue, liver metabolic function and liver lipid peroxidation. Since the study only contained one replicate per cultivation system, it was not possible to extrapolate to the population of organic and conventional foods, but this issue is addressed in an ongoing project (http://www.icrofs.org/).
In another recent study in search of biomarkers, two generations of chickens were fed from two production systems in a farm-pair approach, of which the existing

Table 3 Feeding experiments with feeds from organic *vs.* conventional growing systems

Author	Feed	Animal	Parameter	Results for the group with feed from organic production
Gottschewski (1975)	Common beets, hay, oats, carrots, wheat	rabbits	Reproduction	Biodynamic, significantly fewer perinatally dead and more weaned pups
Edelmüller (1984)	Potatoes, common beets, barley, oats, carrots, beet root	Rabbits	Reproduction	More pups born, fewer perinatally dead, more weaned pups
Staiger (1986)	Dried grass meal, wheat, oats, barley, soya beans	Rabbits	Reproduction	More pups born and weaned, higher weight at birth and during weaning time
Plochberger (1989)	Dried grass meal, wheat, oats, barley, soya beans	Chicken	Egg quality	Significantly higher egg weight
Velimirov and Plochberger (1992)	Barley, oats, soya beans, peas, carrots, common beets	Rats	Reproduction	Significantly fewer perinatally dead pups, better weight gain in lactating females

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differences in nutritional quality, mainly 10% more protein in conventional grain, were maintained (Table 4).[44]

The results of this study still await publication, but here differences in weight gain and immune responsivity were found.

Another multigeneration study on rats has been conducted from 2006 within the EU project Quality Low Input Food.[45] In this study rats were given organic, conventional and two kinds of 'low-input' diets (Table 4).[45] Splenocyte proliferation was used as a sensitive measure to detect potential impacts of diets on the immune system of rats. The proliferation of splenocytes from adult rats appeared to be suppressed when diets were based on crops grown with mineral fertiliser inputs. It suggested that the immune system of rats fed with organically fertilised diets was probably better prepared to fight against infections. The studies are currently continued.

Table 4 shows feeding experiments with the aim of defining health biomarkers.

Feed acceptance, avoidance and preference (food preference tests)

An integral part of feeding experiments is the feeding behaviour of animals. The quantity of feed intake is regulated by the need to keep up energy homeostasis and also depends on the quality of the feed offered. Thus, if a feed is deficient in any nutrients, more will be consumed.[46] In natural surroundings animals can choose their feed according to need, which has led to the development of very complex feeding behaviour that enables animals to recognise 'healthy' foods or balanced diets. Food preference tests are based on this nutritive wisdom. The normal diet should be provided during the test to avoid nutrient deficiencies and the subsequent behavioural disorder. It has to be kept in mind that in organic quality research the objective is not to investigate dietary specifics but to reveal quality differences depending on production methods.

So far, it has been postulated, that for animals to be able to choose, at least one of the offered foods has to be nutritionally unbalanced, otherwise there would be no benefit in choosing. But a number of food preference tests conducted with different animals have shown that even in cases of no analytically apparent imbalances significant preferences have taken place when the test variants originated from different cultivation systems.[47,48]

Similar to most of the above-mentioned feeding experiments, the first food preference tests were also conducted with products grown with different fertilisers. Laboratory mice significantly preferred organic as compared to mineral-fertilised wheat.[49] Later on, food preference tests were concerned with comparing products from organic and conventional production systems. Rabbits and chickens were both capable to distinguish between differently grown feed and significantly preferred organic common beets, potatoes and cereals over the conventional variants.[40,50] Since the 1990s, a number of food preference tests have been carried out with laboratory rats

Table 4 Feeding studies with the aim of defining biomarkers for health effects and disease risk usable for future human studies measuring differences between organic and conventional diets on health effects and disease risk

Author	Objective	Products integrated in complete diets	animal	Health biomarkers	Results
Lauridsen *et al.* (2007)	Factorial design Organic fertilisation/no pesticide Organic fertilisation + pesticide Mineral fertilisation + pesticide	Potatoes, carrots, peas, kale, apples, rapeseed oil, Freeze-dried	Rats	Clinical health and disease status Bioavailability of nutrients and metabolism Physical activity Functions of organs, intestine Analyses of nutritional status in blood and tissues Analysis of immune response Post-mortem evaluation of organs	Diet with organic fertilization/ no pesticide: significantly higher plasma IgG, vitamin E, less body fat and more relaxed
Barańska *et al.* (2007)	Factorial design Organic fertilisation/no herbicide Organic fertilisation + herbicide Mineral fertilisation/no herbicide Mineral fertilisation + herbicide	Barley, potatoes, carrots, onions, pellets	Rats	Analyses of immune response splenocyte proliferation Feed intake Blood morphology Anti-oxidative status of plasma	Proliferation of splenocytes appeared to be suppressed with mineral fertiliser inputs, significantly higher anti-oxidative status of plasma in rats on feeds without pesticides
Huber *et al.* (2007)	Systems approach: organic vs. conventional growing systems	Wheat, barley, triticale, peas, maize, soya, ground	Chickens	Weight development, growth Feed intake Clinical health. disease status Egg production Organ function of different organs by metabolomics and genomics Immune responsivity Response to a KLH-challenge Post-mortem evaluation.	Organically produced feed: significantly lower weight, stronger immune responsivity, stronger catch-up growth and liver metabolism after challenge

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displaying a manifold and flexible feeding behaviour. The instinctive diet selection of laboratory rats can give valuable information about food quality that, so far, cannot be obtained from traditional laboratory techniques.[51] Thus products with no significant content differences were still differentiated on highly significant levels by the rats.[52].
The limitations of the method are determined by inborn taste preferences, e.g. for sweet taste and aversions, such as against bitter or sour. To make the full use of the deep-rooted and well-developed feeding behaviour, it is preferable to test foods that wild rats would sample. Furthermore, individual products should be offered, not whole diets, again simulating natural conditions as closely as possible.
Food preference tests with laboratory rats have also been used to investigate whether they are capable of differentiating whole diets composed of differently grown products.[53] In this study the influence of the mother's diet on food choice could be corroborated, but the choices were different from one individual to another and were changed from one day to another. Thus a preference for organic food could not be established on a significant level. This could be due to offering complete diets instead of single raw products, where post-ingestive consequences are more easily attributed to the test food.
Summarising all food preference tests with laboratory rats conducted so far show that products of marketable quality - from organic as compared to conventional production systems - are significantly preferred. The emphasis on 'optimal' production is important, since laboratory rats choose good quality not production system, implying that growing problems entailing a less than good product quality influence the selective behaviour in all cases.

Feeding trials as models for human health research

The results of feeding experiments in the organic field can also be used to optimise production methods if focused on agricultural aspects or to indicate nutritional effects to optimise farm animal feeding strategies. The main purpose of the feeding trials, however, is to ultimately use animals as models for humans. The extrapolation of such animal-based findings to humans has given rise to controversial discussions. On the one hand, it is maintained that extrapolating results obtained from one species to another involves unscientific speculation: on the other hand, all established safety values concerning residues originating from agricultural and veterinary practice are based on animal feeding trials.
In the context of organic quality research, feeding trials are expected to clarify the question of whether production methods could have health-promoting effects. In the last decades, the consumer motivation to buy organic food has shifted from environmental to health concerns.[11]

The environmental advantages of organic farming are well documented and established. The perceived health benefits are based on results from feeding trials concerning fertility parameters and food preferences as described above, but there is still an urgent need to define more specific health biomarkers, relevant to humans, which connect to the specific approach of organic farming. In the perspective of Lady Balfour's vision of 'Healthy soil, healthy plants, healthy people', it has to be kept in mind that human 'health' is a multifactorial conception. The World Health Organisation defines health as "a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity" (Preamble of the constitution of the WHO, 1948).[54] In more recent years, as in the salutogenesis model developed by Antonovsky in the 1970s, health becomes considered as a dynamical state of well-being characterised by the physical, mental and social potential to meet the demands of life according to age, culture and personal responsibility.[55, 56] Disease, therefore, would be the lack of this potential.[57] This is consistent with the organic aim to optimise the plants and animals natural ability to be healthy. Human health is then not seen as a state, but a process depending on and influenced by complex interactions between genetic predisposition, environment and society as well as life style. In clinical terms health is regarded as the ability of an organism to efficiently respond to challenges and restore well-being (homeostasis). The concept and understanding of health has shifted from a static description of well-being to a dynamic reaction to stressors and changes. In quality research in the organic field, the understanding of product quality has undergone a comparable paradigm shift from a static substantial to a dynamic process-focused assessment on a systems level.[57] Thus the dimension of time has been added to the study of quality, including reactive and interactive processes. From a reductionist view, potential positive health effects of organic food could be attributed to the reduction of synthetic residues as well as the potential increase of health-promoting compounds. From a more dynamic view, it could be hypothesised that the homeostatic state of the food product could be attributed to the strengthening of self-regulating properties of the consumer.

It is recognised that nutrition plays an important role in promoting or reducing the organism's ability to cope with potential health threats as well as maintaining a state of well-being. Nowadays, cognitive function and behaviour testing are increasingly included in nutritional research. The most important influence of nutrition is based on diet composition, but the quality of the consumed products can have a modulating positive effect. So far, this notion has been attributed to the reduction of synthetic residues as well as the potential increase of health-promoting compounds. But the outcome of the cited feeding studies comparing test diets of the same nutritive value, only differing in the way of production, necessitates an extension of the quality concept as described, and entails the application of new quality assessment methods.

The interpretation of the results in the consuming organism on a systems level could be facilitated by including modern systems biology methods like metabolomics which, combined with advanced statistical as well as thorough physiological knowledge, can provide insights at regulatory levels.[58] The challenge for the future will be to combine these methods with the earlier described holistic methods, in development to assess holistic qualities in food products, to search if the hypothesised homeostatic properties of organic food products can be correlated with a balance or imbalance in the consuming organism.

Not all parameters investigated in feeding trials with the described designs are useful biomarkers to be extrapolated to human health. Advantages concerning litter size or food preference provide insight into differences caused by production methods but are less suitable as markers for human health. More recent feeding trials investigating effects on the immune system and nutrigenomic influences can be seen as pioneer studies testing health parameters more directly indicative for human health issues. Thus the aims of future animal feeding trials are the definition of health indicators and the establishment of biomarkers as a basis for intervention studies in humans.

Conclusion of the main characteristics of feeding trials in the organic context, which are guidelines for future projects:

1. Focus either on controlled production methods or on best practice production systems and be clear about the difference.
2. Perform whole food studies in contrast to purified diets.
3. Pay attention to different processing steps in diet preparation.
4. Compose diets according to need.
5. Include modern holistic techniques to approach whole product and whole body concepts.

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Chapter 8

The challenge of evaluating health effects of organic food; operationalisation of a dynamic concept of health

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Abstract

The health benefits of consuming organically produced foods compared with conventional foods are unclear. Important obstacles to drawing clear conclusions in this field of research are (1) the lack of a clear operational definition of health and (2) the inability to distinguish between different levels of health using valid biomarkers. In this paper, some shortcomings of the current definition of health are outlined and the relevance of integrating a more dynamic and functional component is emphasised, which is reflected by the ability to adapt. The state of health could then be determined by challenging an individual with some form of stressor and by subsequent quantification and evaluation of the coherence in recovery of various physiological processes and parameters. A set of relevant parameters includes the activity of the immune system and the activity of the autonomous nervous system. A good recovery towards homeostasis is suggested to reflect a qualitatively good state of health. Furthermore, it would enable objective evaluation of health-optimising strategies, including the consumption of organically produced foods that aim to strengthen health.

Keywords: health; organic food; ability to adapt; homeostasis; allostasis; salutogenesis; resilience; robustness; stress; challenges

Introduction

The market for organic food products is steadily growing, partially owing to a widely held consumer belief that organic foods are naturally produced, safe and healthy. Especially this latter aspect, the expected 'healthiness' of organic foods, is a highly controversial topic within the scientific community, primarily owing to varying results of nutrient comparisons of products and secondly because of the small number of reliable scientific studies assessing potential health benefits of consumption of organically *vs.* conventionally produced food products. As with research on health effects of functional foods, it is difficult to prove the potential health effects of organic food products. These difficulties arise largely from a problem at the basis of nutritional research, namely the need to prove that consumption results in 'an improved state of health and well-being and/or reduction of disease'.[1] To measure health status, reliable (physiological) markers or biomarkers are needed. Markers will be most readily available to evaluate the reduction of diseases, since, in a variety of diseases, different stages can, in principle, be measured quite easily. In this respect, effects from organic dairy consumption on the reduction of allergic symptoms have indeed been reported.[2] More challenging is to define 'an improved state of health and well-being', as objective standards or parameters for different stages of healthiness are currently missing. Owing to the lack of an operationalised definition of health, the question of relevance is how to define 'health' accurately. The current WHO definition, describing health as 'a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity',[3] is increasingly being criticised, partly because of the problem of operationalisation. Recently a proposal has been published for a new concept of health that does not describe an endpoint, like the WHO definition does, but is more dynamic and functional.[4] This formulation of 'health as the ability to adapt and to self-manage' seems to offer possibilities for the development of reliable biomarkers for various levels of health. Moreover, it connects to the principles of organic agriculture of striving to maximise resilience, robustness and adaptability in plants and animals in order to increase their health and to avoid the need for pesticides and antibiotics.[5] Therefore this approach might be the most appropriate in evaluating health effects from organic food.

In this paper we connect this new dynamic and functional concept of health to the physical and physiological domain, namely the ability to adapt, and propose directions for operationalisation. Focusing on this domain will allow for the classification of various levels of health, even among people who are generally considered to be healthy. Such a classification would provide possibilities for a more objective evaluation of the health effects of nutritional strategies, with the consumption of organic foods as a prime example. We will indicate physiological parameters that we consider to be relevant measures of this ability to adapt, such as the activity of the immune system and the activity of the autonomous nervous system. Assessing and qualifying such parameters following a 'health challenge' could provide valuable information about the state of health of an individual.

Organic food *vs.* conventional food

Organic food products are, by definition, distinct from conventional food products owing to the usage of organic production methods, which exclude the use of synthetic fertilisers, crop protectives and preventive antibiotics as well as the use of genetically modified seeds, synthetic additives and irradiation.[5,6] Consumers appear to value organic foods owing to this difference in 'process quality', the way organic foods are produced, processed and how they affect the environment, and in 'product quality', the differences in food properties of organic foods compared with conventional foods.[6]
In recent years it has been frequently reported that there are indeed differences between organic food and conventional food with respect to product quality. Several reviews report organic food products to contain higher levels of vitamins and minerals (among which vitamin C, iron, magnesium and phosphorus), whereas lower levels of nitrate are present. Similarly, levels of phytonutrients such as polyphenols, carotenoids and flavonoids have generally been found to be higher in organic foods, whereas lower levels of undesired compounds such as pesticides and equal or lower amounts of mycotoxins have been observed.[7,8] However, these results are being disputed by other authors, the main and most recent one being a review by Dangour *et al.*,[9] who describe more phosphorus, higher acidity and less nitrate in organic products, but no other differences. In their view the small differences in nutrient content detected are biologically plausible and mostly relate to differences in production methods. The authors did not, however, include most of the studies presenting data of well-controlled field trials.
There exists a big variation in the nutritional content of various organic food products because of e.g. differences in cultivation systems and production methods. Yet there are *indications* that consumption of organic food affects health differently than consumption of conventionally produced food.[10,11] However, *sound scientific evidence* supporting this effect is currently lacking, as not enough well-designed consumption studies suitable to show such an effect have been performed.
Nevertheless, for consumers the health argument is one of the most important reasons to buy organic products, and thus the scientific question whether consumption of organic food indeed results in better health (maintenance) remains highly relevant.

Challenges in researching health effects of organic foods

Studying the health effects of foods is challenging, but studying organic foods is even more challenging. An important difficulty relates to the large diversity among organic food products. The nutritional content of food is influenced by both genetic and environmental factors and is dependent on a variety of aspects, including soil quality (e.g. the time the soil has been worked organically), crop rotation, geographical area, orientation towards the sun and farm management skills.[7,10,11] Recently, Brandt *et al*.[12] described the influence of production methods on plant composition and showed an inverse relation between increased plant available nitrogen (fertiliser) and reduced production of plant defence-related secondary metabolites and vitamin C, compounds which in humans are considered to be health-promoting.

Besides this, product processing and even governmental regulations play a role in the composition of organic foods.[10] Together, this results in a big diversity among organic food products, complicating the assessment of whether organic foods are beneficial to health. After consumption the question of the bioavailability of nutrients following digestion becomes relevant, as well as the bioactivity of nutrients exerting their physiological effects in the body. The availability and effects of these nutrients, although rather unpredictable, are the most relevant factors concerning health effects. Thus health effects of food are best studied following consumption in well-controlled human intervention trials, controlling for, among others, the effects of age and of inter-individual variety in basal parameters. Here we will address concepts and physiological parameters that might help address this problem.

In order to quantify possible health effects, these need to relate to an appropriate definition of health. As Niewold[1] pointed out, health effects might be 'an improved state of health and well-being and/or reduction of disease'. Yet, in their review about health effects after consumption of organic food, Dangour *et al*.[13] took relevant health outcomes as effects on defined diseases and concluded that evidence for health effects was lacking. In their research, van Ommen *et al*.,[14] however, point out that defining health as a condition in which disease is reduced or absent is inadequate for three reasons: (1) processes involved in disease and disease progress are not necessarily the same as those involved in the optimisation of health or prevention of disease; (2) homeostasis regulates various functional biomarkers within a limited range, thereby masking possible early effects or predispositions to disease under 'normal' or 'resting' conditions; (3) inter-individual differences within 'normal' values can vary significantly. Even within an individual, 'normal' values can fluctuate, thereby thwarting the expression of health in a generalised numerical value.

To conceptualise what, then, exactly is encompassed by improved health is a complicated task. Health is affected not only by physical factors but also by psychological, social and environmental factors. A further complication is the fact that health can be explained at different levels, e.g. from a molecular perspective, from an organ or an organism perspective or from a population, a societal or even an ecological perspective. Additionally, health is not marked by clear boundaries and, as stated by Antonovsky,[15] should be considered more as 'a movement in a continuum on an axis between total ill health (dis-ease) and total health (ease)'. It is thus clear that defining health merely as a state in which disease is absent is too simplistic. As will be emphasised in the remainder of this paper, the formulation 'health as the ability to adapt'[4,16] could help to make health effects better measurable and assessable and could aid in classifying different levels of health, thus facilitating nutritional research.

Physiological mechanisms underlying health as the ability to adapt

In order to obtain a full insight into health and to comprehend why the capacity to adapt is such an important aspect of health, one should first aim to clarify and understand the various physiological mechanisms underlying health. Two processes involved in determining a well-adapted and stable physical state are *homeostasis* (stability through constancy) and *allostasis* (stability through change). Both processes are used by the body to achieve *salutogenesis* (origin of health), *resilience* (positive adaptation) and *robustness* (functioning despite disturbances).

Homeostasis

Homeostasis is the concept that underlies a stable physical state in which bodily mechanisms interact in response to various stimuli. The interplay between these mechanisms acts to maintain systemic parameters that are essential for life within a narrow optimal range, thereby maintaining a physiologically stable state. Such essential parameters include pH, osmolarity, glucose levels and oxygen tension. To fully comprehend the concept, the various factors that underlie or influence the different homeostatic mechanisms should be considered. Examples are genes, proteins, chemical composition of body fluids and environmental and social interactions. These are factors that clearly do not act in isolation. For a better understanding of the concept of health, evaluation of the response of homeostatic processes to stress conditions is required.

Allostasis

Compared with homeostasis, a seemingly opposing model called allostasis (stability through change) has gained wide acceptance. The allostasis model does not consider an unusual value as a failure of maintenance of a certain setpoint; rather, it sees it as a response to a certain (anticipated) condition or stressor by adapting the setpoint (Figure 1). Albeit seemingly contradictory, allostasis actually complements homeostasis, as it utilises the process of change to eventually reach a new physiologically stable state that is better adapted to the changed environment. In other words, allostasis is a dynamic process that is responsible for keeping the physiological mechanisms involved in maintaining homeostasis (which is essential for life) in balance. This process is energy-consuming and, if challenged too much, the result of overexposure can be damage described as *allostatic load*.

In the determination of health and shifting into a context of disease, the effects of allostasis can be measured in terms of allostatic state and allostatic load. The distinction between allostatic state and allostatic load lies in the type of endpoint that is measured: allostatic state focuses on the response profiles of mediators themselves, whereas allostatic load refers to tissues and organs showing the cumulative effects of overexposure to mediators of allostasis.[17] Examples of such mediators are adrenalin, glucocorticoids and cytokines, which produce reactive and adaptive responses.[18] This is reflected in possible examples of allostatic states, such as elevated levels of inflammatory cytokines, elevated and flattened diurnal cortisol rhythms, elevated overnight urinary cortisol and overnight urinary catecholamines.[17] Allostatic load results when prolonged activation of allostatic responses causes a cumulative pathophysiological change (secondary pathophysiological outcome).[18]

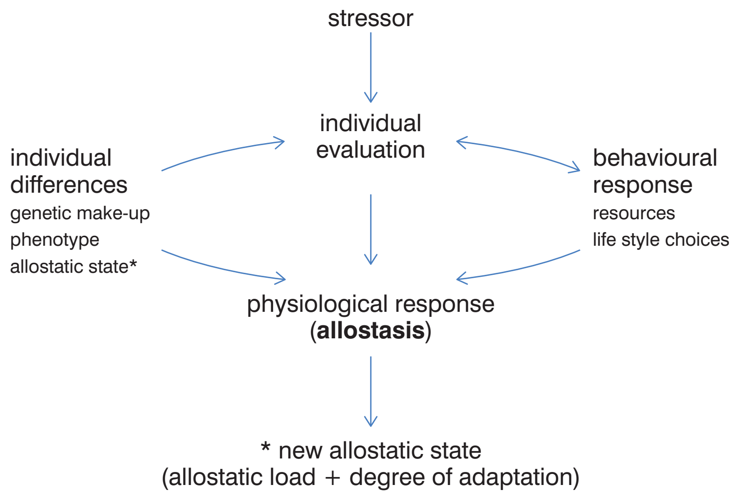

Figure 1 Allostasis

Allostasis can be depicted as a system with on the input site, the stressor, which can differ in strength and quality and can be either an external disbalancing impulse or an internal disturbance in the regulatory network of allostasis. The evaluation of this stressor depends on the type of stress, the intrinsic factors like the genetic and phenotypic make-up and current allostatic state (individual differences), as well as the extrinsic factors including the resources available and the life style choices made (behavioural response). When a threat to homeostasis is perceived (individual evaluation), a behavioural and physiological response (allostasis) is initiated. Allostasis leads to a new allostatic state that may influence future responses to stressors. Adapted from McEwen.[44]

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Examples that reflect allostatic load are, in order of increasing severity, abdominal fat deposition, atherosclerotic plaques and neuronal atrophy or loss in the hippocampus.[17] Specific biomarkers that can be used as measures of allostatic load in response to different types of stress are described by Juster *et al*.[19]

Salutogenesis

Upon stressors the body initiates a wealth of repair and adaptation processes to maintain its inner balance via either homeostatic or allostatic processes. Generally, a stressor can be defined as a threat to homeostasis – a force attempting to alter a static condition.[17,20] Even though the repair and adaptation processes acting to cope with stress do not always occur without damage, the body deals with most stressors without significant problems. To explain why people, despite experiencing stressful situations, manage to stay perfectly healthy, the concept of salutogenesis ('origin of health') has been developed.[15] Salutogenesis includes two key elements: (1) an orientation towards problem solving and (2) a capacity to utilise the resources available.[21] The concept can be described as a coping strategy by the body in which the psyche, the central nervous system and the immune system together influence in interaction the physiological response to a certain challenge with the aim of maintaining general physiological health.

Next to salutogenesis as a coping strategy, there are two main concepts in the literature regarding the sensitivity of systems towards perturbations: resilience and robustness. The concept of resilience was developed and explored in social and ecological systems, emphasising the aspect of recovery following disturbances, whereas the concept of robustness was developed in engineering and biology, emphasising the aspect of maintenance of function despite suffering stressful conditions. The two lines of thought are not mutually exclusive and can be used as equivalent terms for different domains.[22] These two adaptation processes and their relation to different types of stressor are explained in further detail below.

Resilience

Resilience or elasticity is described by Cicchetti and Blender[23] as 'a dynamic developmental process that has been operationalised as an individual's attainment of positive adaptation and competent functioning, despite having experienced chronic stress or detrimental circumstances, or following exposure to prolonged or severe trauma'. This implies that the variable outcomes of stress responses can be explained by an individually based underlying mechanism. Analogously to salutogenesis, various psychosocial factors have been associated with stress resilience. These include (1) the use of active coping strategies such as problem solving and planning, (2) optimism and high positive emotionality, (3) positive reframing and acceptance, (4) social competence and social support and (5) a sense of purpose in life, a moral compass and spirituality.[24] Neurotransmitters, neuropeptides and hormones are assumed to play an important role in salutogenesis as well as in the physiological processes underlying resilience.

Robustness

Analogously, robustness indicates that an organism (or a biological system) can function despite the presence of internal or external disturbances.[25] Important here is the maintenance of function and not the maintenance of homeostasis as such.[26] Consisting of a control system with many feedback loops, robustness is often seen as a fundamental property of each evolved complex system in which subsystems may contribute to the homeostasis of the overall system.[25] It depends on the type of disturbances (challenges) whether a system is evaluated as either robust or relatively weak. For example a bacterium can be evaluated robust (resistant) against threats from antibiotics but (because of having extra genes to replicate) be increased vulnerable against shortage of resources.[26] In this view, disease is an overt sign of weakness of a certain system to a specific disturbance. It is good to realise that it is not easy to improve the robustness of a system as a whole (and thereby indirectly an aspect of health of the individual), since an improvement in robustness to a specific disturbance might either lower robustness in other areas, heighten weakness to other disturbances or increase energy requirements.[25] Robustness, in that sense, neatly illustrates the interrelated dynamics of the maintenance of a state of health and is a valuable concept in the operationalisation of a functional concept of health.

Health as a functional and measurable concept; the effect of different stressors

From the above-described physiological processes involved in maintaining overall health, we can deduce a common theme: they are all physiological responses towards stress (a disturbance of homeostasis) in which the body tries to cope with an imbalance induced by an encountered stressor. The capacity and execution of these physiological responses can hence be viewed as functions of health. Many of these physiological responses can, via differential parameters, be adequately measured and quantified, providing potential important information on health status. The adaptability of the body to specific stressors is therefore an important operational aspect of a new concept of health.

To make this functional component of health usable in research, the concept needs to be translated into quantifiable physiological responses upon specific and standardised stressors. In order to do this, the type of stressor that can activate the above-mentioned processes should first be identified. Such a stressor can then be used to destabilise the system, after which the recovery process can be measured. The speed and extent to which this recovery takes place can then be evaluated as a possible measure of the health status. Here we briefly discuss the different types of stressor, the parameters for physiological response that can be used, as well as the path to recovery and the extent to which the combination of the different physiological processes can be used as a functional measure of health.

Oxidative stress

Research has shown that exercise can lead to an increase in cellular free radical quantity.[27] To compensate the potentially harmful effects of these free radicals, the antioxidant-producing capacity of the vascular wall is stimulated.[28,29] Generally speaking, when this adaptive response is adequate, a state of vascular balance is maintained or even an improved state is achieved. However, whereas mildly elevated free radical levels (via intracellular signal transduction pathways) might result in a successful adaptation to stress, heavy training of extreme intensity and long duration might result in free radical levels that can no longer be neutralised by the cellular antioxidant defence.[27,30] This can result in permanent tissue damage, which is an example of allostatic load that impedes recovery mechanisms.

Cardiovascular stress

To adapt to increasing demand for oxygen (such as during exercise), an intricate interaction takes place between the sympathetic (nor)adrenergic system, the endocrine system and the cardiovascular system. Together, the activation of these systems augments the pumping function of the heart. Parameters that reflect this complex interaction have an important prognostic function.[31] Examples of such parameters are heart rate (HR), heart rate recovery (HRR) and heart rate variability (HRV), which can be measured both in rest and in response to exercise[32] as well as in relation to many other stressors. It has been shown for example that trained individuals have a lower basal heart rate than people with a more sedentary lifestyle.[32] Similarly, fit people have, dependent on their training intensity, higher peak heart rates.[33] In an experimental setting, oxidative and cardiovascular stress could be induced by exposing a subject to e.g. an exercise test. This test could, depending on the aim, be short and intensive or longer and less intensive. Assessing the changes in these parameters during both exercise and recovery may therefore provide information on the health status of an individual.

Immunological stress

The effects of stressing the immune system are complex and involve multiple intricate pathways. Immunological stress is associated for example with the induction of antigen-specific cell-mediated immunity as well as long-term events in immunological memory.[34,35] These immune-stimulating effects facilitate an adaptive response to a mild stressor. Chronic or extremely intense stress to which no adequate response is mounted can, however, result in an immunosuppressive effect.[36–39] This delicate balance between immune-stimulatory and immune-suppressive effects of stress shows the intricacy of the body as well as of the concepts discussed in the previous section. To quantify an effective immunological response to stress, parameters should thus be chosen with caution. For example, in the case of (acute) stress, changes in levels of circulating hormones such as noradrenaline and adrenaline (and their derived products) are important,[40] as are changes in levels of hormones from the HPA (hypothalamus-pituitary-adrenal) axis such as ACTH (adrenocorticotropin hormone) and cortisol. These hormones are involved not only in homeostatic physiological mechanisms but also in the regulation of the immune system. Fluctuations in concentrations of these hormones can therefore be used to measure the extent of the adaptive response as well as the interaction of the stressor with the immune system.[41] In a nutritional context, then, various biomarkers could be used to analyse the effects of such a stressor, as extensively described by Albers *et al.*,[42] who list various immune biomarkers in terms of their usability.

Immunological stress in an experimental design can be induced by the injection of e.g. an attenuated vaccine. A stressor load that is relatively too strong or that yields an insufficient response could lead to chronic inflammation, which can be regarded as allostatic load at the level of the immune system.

Psychological stress

Several brain structures are involved in the initiation of the adaptive response, among which are the prefrontal cortex, the amygdala, the hippocampus and the hypothalamus. These brain areas are, via feedback loops, intricately connected with each other. The prefrontal cortex, which is involved in planning and execution, has an important role in the regulation of this response: via GABA (γ-aminobutyric acid), this brain area normally inhibits the amygdala, the area involved in emotions such as fear, anxiety and aggression. Also, the hypothalamus, the hormonal regulation centre, is inhibited by the prefrontal cortex. Under circumstances of insecurity or threat, the activity of the prefrontal cortex might decrease.[43] Owing to this lowered activity, the amygdala and the hypothalamus are activated, resulting in the release of catecholamines. Similarly, the activity of the SAMS (sympathetic adrenal medullary system) increases, initiating the famous fright-fight-flight response. This results in the release of CRH (corticotropin-releasing hormone), stimulating the release of cortisol via the HPA axis and increasing the production of cortisol.

The hippocampus, which is responsible for memory and selective attention, plays an important role in negative feedback by reducing the stress response.[44] The stress response and the release of several stress hormones aim at adapting towards a potential new stressor. However, when a chronic imbalance arises in the regulating mechanisms, which can be caused by e.g. a lack of negative feedback, the health status can become affected and a pathological situation might arise. A chronic hypoactive prefrontal cortex can be involved in psychopathologies such as severe anxiety, depression, post-traumatic stress syndrome, burn-out or schizophrenia. A slow reaction towards new, neutral stimuli and impaired functioning of the working memory can be explained similarly by a hypoactive prefrontal cortex that will no longer inhibit the amygdala and the hypothalamus.[45] Lastly, an impaired feedback circuit might result in higher levels of circulating cortisol. This might lead to a build-up of allostatic load, as high cortisol levels are associated with a suppressed immune system and physiological stress. The allostatic state is also reflected in high levels of interleukins, which might contribute to low-grade chronic inflammation,[45] associated with pronounced metabolic changes such as insulin receptor insensitivity, reduced glucocorticoid receptor sensitivity, high circulating proinflammatory cytokines and activation of the glutamate system, resulting in high levels of blood glucose, which might cause an increase in the number of advanced glycation end-products (debilitating the elasticity of the vascular wall and stimulating the aging process). All these parameters increase the allostatic load.

From the previous paragraphs it is clear that there are different types of (physiological or psychological) stress conditions, resulting in a variety of responses by the different organ systems. These responses are closely regulated at the cellular, organ and systemic or organism level. Various physiological parameters that can be used to express the responses to different types of stressors were described, including antioxidant capacity by the vascular wall, heart rate (variability) and heart rate recovery, levels of circulating stress hormones as well as levels of interleukins and cytokines. How these different parameters are connected is illustrated in Figure 2. Following, in Table 1 an overview is provided of these parameters and physiological responses measured previously in human stress studies, as well as other relevant biomarkers that can potentially be used in nutritional studies on the possible beneficial effect of organic food products. Interestingly, these parameters have been used in human stress studies but have hardly been explored as such in nutritional research.

Towards a functional measure of health; selection of relevant biomarkers/parameters

As can be deduced from Table 1, there are a large number of factors that underlie the proper functioning of homeostatic and allostatic processes and therefore the concepts of salutogenesis, resilience and robustness. To be able to quantify health and the effects of e.g. organic food products on general health in terms of adaptability, a group of markers needs to be selected. Together, these markers should be able to provide a good indication of the dynamic state of a person and the extent to (and speed with) which a person is capable of recovering from stressful conditions.

Multiple aspects of health could then be quantified by letting an individual undergo several challenges or 'stress tests' such as exercise, a psychological challenge or an immune challenge. At that point, allostatic load, allostatic capacity, resilience and the process of recovery can be objectively quantified. To measure allostatic load, levels outside the normal range will be most informative (e.g. night blood pressure). To measure allostatic capacity, on the contrary, one should compare levels indicative of a static homeostatic state with those during a stress test. These data would then be illustrative for disturbances in homeostasis. For a more dynamic image, one could determine a set of biomarkers measured before, during and after various periods of time following a physiological (or psychological) stress test to determine the time needed to recover from a specific stressor. Measurement of the time component has the additional advantage that one can calculate the area under the curve, which

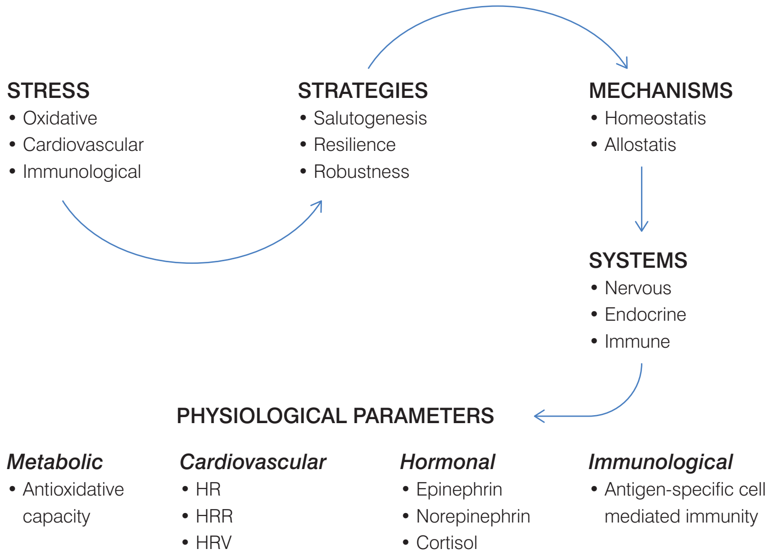

Figure 2 Stress Responses

Different types of stress activate a salutogenic or resilient response, of which homeostatic and allostatic processes form the basis. Here, a coherent, integrated response between the different organ system takes place. A successful stress response can be determined via several parameters in an experimental setting. (e.g. CFVR, coronary flow velocity reserve; LAD, left anterior descending artery; HR, heart rate; HRR, heart rate recovery; HRV, heart rate variability; DTH, delayed-type-hypersensitivity).

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Table 1 Physiological Parameters to Measure Stress Responses (Adapted from: Steptoe[46])

Parameter	Physiological Response
Musculoskeletal	Muscle tension, breathing patterns
Neuroendocrine	Corticosteroids (cortisol, mineralcorticoids), catecholamines (adrenaline, noradrenaline), b-endorphin, testosterone, prolactin, growth hormone, insulin, DHEA
Cardiovascular	HR, HRR, HRV, cardiac arrhythmias, cardiac output, stroke volume, myocardial contractility, pulse transit time, blood pressure, total peripheral resistance, regional blood flow (in muscle and skin), coronary blood flow, transient myocardial ischemia, renal blood flow, glomerular filtration rate, sodium excretion, platelet aggregation and adhesion
Electrodermal	Sympathetic nerve activity (microneurography), skin conductance, skin potential, sweat gland counts
Gastrointestinal Tract	Salivation, gastrointestinal transit time, electrogastrogram, fat metabolism, total cholesterol, cholesterol fractions, triglycerides, free fatty acids, blood glucose levels
Immune-Related	Immunoglobins (IgA, IgE, IgG, IgM), lymphocyte subsets, natural killer cell activity, mitogen-induced lymphocyte proliferation, antigen titres to latent Epstein-Barr virus, interleukins, cytokines
Blood Serum	Creatine, albumin, uric acid, heat shock proteins

would indicate the intensity of the bodily reaction.[14] The analysis of a set of relevant parameters reflecting the activity and response to a stress-induced imbalance should additionally be focused on the coherence of recovery of these parameters reflecting different aspects of the autonomic nervous system and/or immune system. A well-coordinated pattern of recovery towards homeostasis might be suggested to reflect a qualitatively good state of resilience and health.

Important considerations for such a model relate, among others, to the determination of the border values that will indicate if a level or value is within the ‘healthy’ or ‘unhealthy’ range. Lastly, statistical analysis of the data might be challenging owing to the number of factors measured and the great inter-individual variety. These can be partially circumvented by the utilisation of -omics research (e.g. metabolomics, proteomics, genomics), which might, in future times, allow for personalisation of the results obtained.[14] The development of algorithms might help to circumvent these challenges.[47] In any case, measurements need to be performed under well-controlled circumstances, as health status (and its measurements) can be highly dependent on many factors. Such studies will, at least in the near future, be costly, as routine procedures with validated reference values have not yet been fully established, although research in this area is increasing.[48–51]

Evaluating health effects from organic food in animals and humans

Recent animal studies have tried to apply the quantification of several of these health parameters in feeding trials with organic products. Studies by Lauridsen *et al.*,[52] Baranska *et al.*[53] and Huber *et al.*[54] analysed the effects of either a single food component or whole food organic feeding on parameters in rats and chickens, analogously to the ones mentioned in this paper (blood morphology, organ function, immune response, physical activity).[11] Only limited use was made, however, of inducing a stress response to study the health effects. Huber *et al.*[54] applied an immunological challenge; the resulting differences in immune responses, however, posed difficulties in interpretation. The phenotypical sign of a better 'catch-up growth' after the challenge among chickens on organic feed suggested that the animals could integrate their stronger immune response into a coordinated resilient response. In pediatrics a recovery with catch-up growth after an illness is considered to be a healthy phenomenon (Goudoever H, personal communication, 2011). Yet scientific reference data on such phenomena are still lacking.

The study of Huber *et al.*[54] does indicate the feasibility of applying the concept of adaptation in animal studies to evaluate health effects of nutrition and of organic foods in particular. Determination of the parameters most valuable and most easily measured in animals could help in making a first step towards human organic feeding trials in which health status can be adequately assessed and analysed. Studies with humans should preferably be in situations as controlled as possible, such as among volunteers in a research institute (e.g. students) or among (groups of) individuals in an elderly home, prison or monastery. Individuals should be exposed to challenges (stress tests) of different kinds before and after a start with consumption of organic food. Thus case studies should be gathered in which individuals are their own control. Ideally, participating individuals could be exposed to challenges after increasing periods of time, thus providing information about possible increasing effects in adaptive ability upon prolonged consumption of (organic) food. These types of (pilot) study will not only provide information on a set of values, levels and parameters that are representative of the adaptive ability of individuals but will also provide information on the number of individuals required in future intervention studies.

Conclusion

In order to effectively measure the health effects of organic food products as compared with conventional foods, a dynamic, functional definition of 'health' that allows the evaluation of possible preventive effects of (organic) foods and their potential benefits to otherwise healthy people is preferred. We propose 'health as the individual's ability to adapt to stressful situations' as a first step in the operationalisation of health. Health can then be measured/quantified on allostatic load, allostatic capacity and resilience via various physiological parameters that are changed during a body's stress response. We realise, however, that these parameters will only describe a small part of the health phenomenon. The challenge lies then in the retrieval of a set of values, levels and parameters that might most adequately represent this phenomenon. Such a set of parameters would allow for the assessment of the effect of nutritional strategies such as the consumption of organic foods, as well as for the evaluation of various preventive and/or therapeutic strategies in other areas. Adding 'the ability to adapt' as a dynamic, functional component to the current concept of health might contribute to this quantification and allow for a clearer understanding of the effects of nutrition on health status.

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Chapter 9

Effects of organically and conventionally produced feed on biomarkers of health in a chicken model

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Abstract

Consumers expect organic products to be healthier. However, limited research has been performed to study the effect of organic food on health. The present study aimed to identify biomarkers of health to enable future studies in human subjects. A feeding experiment was performed in two generations of three groups of chickens differing in immune responsiveness, which were fed identically composed feeds from either organic or conventional produce. The animals of the second generation were exposed to an immune challenge and sacrificed at 13 weeks of age. Feed and ingredients were analysed on macro- and micronutrients, i.e. vitamins, minerals, trace elements, heavy metals and microbes. The chickens were studied by general health and immune parameters, metabolomics, genomics and post-mortem evaluation. The organic and conventional feeds were comparable with respect to metabolisable energy. On average, the conventionally produced feeds had a 10% higher protein content and some differences in micronutrients were observed. Although animals on both feeds were healthy, differences between the groups were found. The random control group of chickens fed conventional feed showed overall a higher weight gain during life span than the group on organic feed, although feed intake was mostly comparable. The animals on organic feed showed an enhanced immune reactivity, a stronger reaction to the immune challenge as well as a slightly stronger 'catch-up-growth' after the challenge. Biomarkers for future research were identified in the parameters feed intake, body weight and growth, and in immunological, physiological and metabolic parameters, several of these differing most pronounced after the challenge.

Keywords: Organic food; intervention; chicken model; biomarkers; immunology; metabolomics; nutrigenomics

Introduction

An important reason for many consumers to buy organic products is the assumption that these are healthier than conventionally produced products. However, until now, limited research has been performed to study the effect of organic food on health. Most studies on organic food are dealing with differences in nutrient content of organic vs. conventional products. Organic food is defined as originating from certified 'organic' production according to International Federation of Organic Agricultural Movement standards[1], which exclude the use of synthetic inputs such as synthetic fertilisers and crop protectives, as well as of GM seeds, synthetic additives and irradiation.
Several recent literature reviews have concluded that there are on average higher levels of nutritionally desirable compounds like vitamin C, antioxidants, polyphenols and DM. Conventional grains contain on average higher levels of proteins. Furthermore, equal to much lower levels of undesirable compounds like pesticides, nitrate and mycotoxins are found in organic produce.[2,3,4,5,6,7] The variation in nutritional content of organic products is large, and differences over years within one production system may be larger than differences between production systems.[8,9] Though differences in nutritional content may exist, results from such studies can only speculatively be connected to health effects. Little is known about factual effects of organic food on physiological processes in consumers.
Based on conclusions of an Expert Meeting of the organic Food Quality and Health Association[10], potential effects of organic feed were hypothesised to be found in the immune system of young organisms. It is known that via the gut, food might influence the immune system in the developing organism.[11,12,13] However, a broader exploration of effects, besides immune parameters, was considered to be valuable. Subsequently, a study in rats[14] showed a higher proliferative response of lymphocytes in vulnerable, malnourished animals fed organic wheat, than in those fed conventionally produced wheat. Another rat study[15] compared three isoenergetic and isonutritious feeds from products derived from either low fertiliser input without pesticides (described as 'organic'), low fertiliser input plus pesticides, or high fertiliser input with pesticides (described as 'conventional') and found a higher serum IgG concentration and less adipose tissue in animals fed with the low fertiliser input, both with and without pesticides. Furthermore, the rats fed the 'organic' diet had a higher day time activity compared to animals on the other two diets. Plasma concentrations of oleic and linoleic acids, and ń and ńtocopherol differed between 'organic' and 'conventional' diets, despite similar fatty acid and vitamin E content in the feeds.
These first studies indicate that differences in health parameters may occur as a result of the consumption of feed from different cultivation systems. Before intervention studies can be performed in human subjects, these potential health effects need to

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be explored further and good biomarkers of health need to be defined. The present paper describes the results of a large intervention study to identify biomarkers of the effect of organic feed on health in chickens, focusing on immune responses, innate (humoral: natural antibodies (AB)[16] and alternative complement; cellular: monocytes) as well as specific compartment (humoral: vaccine responses and classical complement; cellular: T-cells), growth and metabolism. It was shown that the response of the chicken fed on either organic or conventional feed showed remarkable differences.

Material and methods

Animals and housing

The study comprised a blinded animal feeding experiment in two generations of chicken fed with feed from either organically or conventionally produced ingredients. National and institutional guidelines for care and use of experimental animals were observed, and the study design was approved by the Animal Welfare Committee of Wageningen University, The Netherlands.

The animals were chickens from the Wageningen Selection Lines, ISA Brown Warren medium heavy layer hens, which were divergently selected during 25 generations for either their primary high (H-line) or low (L-line) AB response to sheep red blood cells immunization at 35 d of age.[17,18] These chicken lines differ in almost every aspect of innate and specific humoral and cellular immunity[19]. Next to these selected chicken lines, a randomly bred control group (C-line) of chickens, resembling the original parental stock, was included. A two-generation design was chosen as it was assumed that due to epigenetic mechanisms, the nutritional status of the mother may influence various physiological parameters of the chicks from the next generation.[20,21] Per line (H-, L- and C-lines) chickens of the first experimental generation of 72 hens were randomly assigned to the organic or conventional feed group. All animals were fed a normal commercially obtained chicken diet till 11 weeks of age. From 11 weeks of age onwards, the animals received identically composed experimental diets, based on either organic or conventional products. Parallel, a group of 22 roosters from the same lines was raised with the experimental feeds (3-4 per group) to produce the second experimental generation through artificial insemination.

The second generation consisted of 150 chickens, divided into six groups corresponding with the H-, L- and C-lines, all with an organic and conventional feed group, to obtain 25 animals per group. The total amount of animals in the second generation was reduced to 145 due to the presence of misclassified rooster chicks. This resulted in the final groups resulting in 22 to 25 animals per line-feed combination; 26 animals being kept when misclassifications became apparent, to obtain an average of 25 animals per line-feed combination.

The second generation of the 6 line-feed combinations received the experimental feeds from hatch till the end of the experiment. Both generations had *ad libitum* access to feed and water. Fresh water was available through a water pipe with drinking nipples.
The first generation of chickens was housed in individual battery cages to ensure identification of the eggs for the second generation. The second generation was housed in spacious and enriched indoor runs (2.28 m^2) in groups of 6 animals, 2 of each line, to minimize the risk of feather pecking. According to routine schemes of Wageningen University, temperature was between 16 and 21 °C, light exposure was increasing from 8 to16 hours and from 500 to 1900 Lux.
Chicken received vaccinations according to commercial schedules[22], though the number of vaccinations was limited as much as possible in order to reduce influencing immune parameters as well as stress. The first generation was vaccinated against Mareks Disease, Infectious Bronchitis, Newcastle Disease (NCD), Infectious Bursal Disease, Pox Diphteria wing web, Infectious Laryngotracheitis and Myoclonia congentia. The second generation was vaccinated against Mareks Disease, Infectious Bronchitis, NCD, Infectious Bursal Disease and Pox Diphteria wing web, and received a dietary anti-coccidium (Paracox-8) due to the risk of coccidiosis connected with floor housing.
At 9 weeks of age, all animals of the second generation received an intramuscular injection in the breast muscle with 1 mg of the non-pathogenic, T-cell dependent protein keyhole limpet hemocyanin (KLH) in 1 ml PBS (pH 7.2) per animal, serving as an immune challenge. The second generation was sacrificed at 94 d (13.3 weeks) of age.

Experimental diets

Feeds were composed of six ingredients: wheat, barley, triticale, peas, maize and soya that were produced either organically or conventionally. Products were obtained according to 'farmer approach resourcing' from neighbouring farm pairs of conventional and certified organic farms, preferably known as 'best practice farms', with the same basic soil and climatic conditions and preferably the same variety per product. Ingredients were obtained from The Netherlands, Austria and Denmark. Before ingredients were used for feed production, they were prescreened for residues of (apolar) pesticides or mycotoxins. Ingredients contaminated with mycotoxins above the maximum residue limit were omitted for feed production. Pesticides were not detected.
Three feeds, a starter, a grower and a layer feed, were composed for the different development stages of the chicken according to existing standards for organic chicken feed[23] by a feed manufacturer (Kruyt, Gouda, The Netherlands). Ingredients were stored at this firm, in dry and dark conditions, at a temperature of 10-17 °C. An

exception was the soya that was stored in Wageningen, where it was toasted prior to further processing, in order to inactivate the antinutritional factors. Every 6 weeks, a fresh badge of feed was produced.

In the different feeds, the ingredients wheat, barley, triticale, peas, maize and soya were represented in different proportions (Table 1). To prevent shortages in the nutritional needs, the feed was supplemented with potato protein, the amino acid methionine, chalk, grid, salt, $NaCO_3$ and a commercial mix of vitamins and minerals. After manufacturing, feed samples were tested on protein composition, in order to prevent shortages of essential amino acids. If a shortage existed, an amino acid was supplemented up to the minimal required level. Other existing differences, either in nutrient content or bacterial load, were accepted as they were considered to reflect reality. The feed was presented to the animals as a composite flour. Feeds were coded either A or B. During the whole project, samples were kept coded so that all persons involved in the study, both in the analyses of feeds and in the feeding experiment, were unaware with respect to the origin of the samples. To avoid any bias, the samples were only deblinded after all results of the analyses of the ingredients and the feeds as well as of the animals were available, interpreted, agreed upon and described in a draft report.

The organically cultivated feed will further be described as ‘organic feed’ and the animals as ‘organic animals’ or ‘organic group’; the conventionally cultivated feed will be described as ‘conventional feed’ and the animals as ‘conventional animals’ or ‘conventional group’.

Chemical and microbial analyses of the feeds

The feeds and feed ingredients were analysed for macro- and micronutrients, i.e. vitamins and trace elements/heavy metals/minerals,bacterial content en endotoxins. All analyses were performed according to Standard Operating Procedures (SOP) at TNO Quality of Life. Analyses were performed before the feed was supplemented with amino acids (see paragraph on the experimental diets), except for two feeds where for logistic reasons this was not possible before supplementing.

Ash content. Samples, after preheating, were heated at high temperature. The residue was weighed.

Total carbohydrates. Samples were rendered soluble in boiling water. Amylum was converted to soluble carbohydrates. The carbohydrates were hydrolysed to mono-saccharides and subsequently analysed by the Luff-Schoorl method.[24]

Raw fibre. Samples were boiled in acid and diluted in alkaline solution. Remaining solid substances were incinerated.

Crude fibre. Samples were hydrolysed with HCl and subsequently extracted with petroleum diethyl ether. The extract was evaporated and the residue was weighed.

Moisture. Samples were dried and weighed before and after drying.

Table 1 Feed composition per age group*

	Starter	Grower	Layer
Age group (weeks)	**0 – 6**	**7 – 17**	**From 18**
Maize	20.00	20.00	25.00
Wheat	30.00	26.42	25.23
Barley	5.00	10.00	5.00
Triticale	12.05	0.00	0.00
Soya beans (heated)	0.00	10.17	19.87
Soya flakes	10.16	20.00	0.00
Peas	10.00	10.00	10.00
Potato protein	7.00	0.00	2.50
MonoCalcFos	1.13	0.73	1.01
FX layers premix†	1.00	1.00	1.00
Fat (plant origin)	1.50	0.00	0.52
Salt	0.07	0.09	0.06
Chalk	1.64	1.16	7.65
Shells (broken)	0.00	0.00	2.00
$NaCO_3$	0.09	0.08	0.00
Methionine	0.11	0.04	0.15

* Numbers reflect the percentage of the ingredient in the total feed. The first generation consumed grower and layer feed, the second generation consumed starter and grower feed.
† FARMIX (Trouw Comapny, The Netherlands)

Protein. The protein content was determined by the Kjeldahl method. Samples were destructed converting organic nitrogen to ammonium. Ammonium was converted to ammonia. Protein content was calculated from the nitrogen-amount.
Fatty acids. Fat was saponified and subsequently transformed to fatty acid methyl esters using methanol and BF_3 (alkaline conditions: NEN-EN-ISO 5509:2000, NEN-EN-ISO 5508:1995). The fatty acid methyl esters were analysed by GC with flame ionisation detector (GC-FID). Quantification was carried out using external calibration of reference compounds.
Amino acids. All proteins in the samples were hydrolysed by boiling in HCl. The resulting amino acids were subsequently separated by ion chromatography, derivatised post-column and quantified using an amino acid analyser[25]. For tryptophan the hydrolysis was carried out with barium hydroxide. Analysis was performed with HPLC with fluorescence detection (HPLC-Flu).[26]
Chloride. Samples were extracted with water and diluted in HNO_3. The amount of chloride (Cl) was determined potentiometrically.

Vitamin E (α-,β-,γ and,δ-tocopherol). After saponification of the sample, tocopherols were extracted and analysed by HPLC with fluorescence detection.[27]
Total folate. Sample extracts were added to culture medium. From the growth of bacteria, the total folate concentration was determined.[28]
Trace elements, heavy metals and minerals (cadmium,chromium, iodine, iron, lead, manganese, selenium and arsenic). Samples were destructed by HNO_3 digestion or incineration. The resulting solutions were analysed either by inductively coupled plasma atomic emission spectrometry (NPR 6425:1995) or inductively coupled plasma MS (EPA method 6020). All experiments were carried out in duplicate. If the duplicate analysis showed a difference larger than 10%, the analysis was repeated. The mean values of the duplo were reported.
For all separate ingredients of the feeds: wheat; triticale; barley; peas; maize; soya, the same analyses were performed. Additionally, bioactive compounds were measured (carotenoids, flavonoids, catechins and phytosterols).
Microbial diversity as well as *endotoxins* were analysed on ingredients and feed. Enterobacteriaceae were measured on a violet red bile glucose (VRBG) agar, incubation at 37˚C for 24 h. Moulds were analysed on Oxytetracycline Gist Glucose Agar at 25˚C for 5 d. Endotoxin in the extracts was measured with the kinetic Limulus Amebocyte Lysate test kit (Bio-Whittaker/Cambrex; Kinetic-QCL; LAL lot no. 3L433E).

In vivo measurements on the animals

General health parameters. Figure 1 presents the time line of the observations and measurements relative to the feeding and age of the first and second generation of animals. The animals were seen by the caretaker daily and abnormalities were registered. Weight and feed intake were measured weekly, onset, and amount of egg production in the first generation was registered.
A routine blood check was performed at 6 weeks of 10 chickens, on *Aviary Influenza, Mycoplasm synoviae, Mycoplasm gallisepticum and Salmonella* spp. No infections were observed.
According to schedule in Figure 1, blood was sampled at five time points in the first generation as well as in the second generation. Moments were chosen before and after 'life events' of the animals, being in the first generation the change from commercial towards experimental feed at 11 weeks of age, and in the second generation the KLH-challenge at 9 weeks of age. Serum and plasma from heparinized blood were collected and stored at -20 °C until further analysis. The second generation was seen at 10 weeks of age by a poultry veterinarian to evaluate general health and feather development.

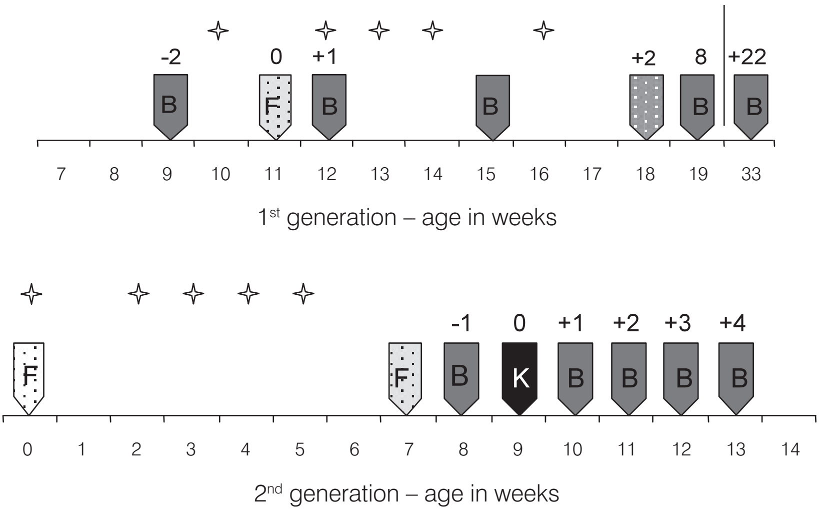

Figure 1 Time frame of first and second generation chickens with vaccinations (✧), original feed (OF), feed changes (F) and blood sampling (B)

At 13.3 weeks of age (94 d), life of the animals of the second generation was terminated by cervical dislocation. The animals were weighed after which they were dissected. The body was inspected for abnormalities and the complete gastrointestinal (GI-) tract from distal oesophagus till cloaca, including the omentum, liver and gall bladder, spleen and bursa was taken out and separated. The liver was separated from the gall bladder, weighed, divided and part was prepared for metabolomics in liquid nitrogen, and part for histological analysis in 10% formalin.

The spleen was divided and prepared for histological analysis and biobanking. The gastrointestinal (GI-)tract, without liver, gall bladder and spleen was weighed after the stomachs had been emptied. For practical reasons the rest of the gut was left filled as it was. The two stomachs, ventriculus and proventriculus, and the bursa were taken off and prepared for histological analysis. From the GI-tract, samples were prepared of duodenum, jejunum and caecum for genomics, as well as for histological analysis. Thymus, heart, lungs, kidneys, pancreas, ovaries and some bone were prepared for histological analysis. Tissues not used in the first round of analyses were biobanked for either histology or genomics. From 36 animals, brains were taken out and prepared in hapteen for biobanking. The rest of the tissue was discarded.

Immunological parameters. Immunological parameters consisted of cellular and humoral components of both innate and specific immunity. In the first generation,

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cellular parameters were analysed at weeks -2, 1, 4 and 8 in relation to the feed change; humoral parameters were analysed at weeks -2, 1, 4, 8 and 22. In the second generation, cellular parameters were analysed at weeks -1, 1 and 4 in relation to the KLH challenge; humoral parameters were analysed at weeks -1, 1, 2, 3, 4.

To determine effects of different feeds on responsiveness in blood monocytes, monocyte activity was measured in a NO assay. Mononuclear cells were isolated from heparinised blood using a histopaque density gradient. In brief, triplicate cultures with 10^6 cells per well were incubated in 96-well flat-bottom plates for 72 h at 4°C with 5% CO_2 with or without (= control) lipopolysaccharide (LPS) in 200 μl Roswell Park Memorial Institute medium. After incubation, 50 μl culture medium was mixed for 10 min at room temperature with 50 μl Griess reagents' in a 96-well flat bottom plate. Extinctions were measured at 540 nm. Monocyte reactivity was calculated using a nitrite calibration and expressed as μM NO production. Levels of natural AB binding LPS or lipoteichoic acid, or specific AB to KLH and vaccines in plasma from all birds at several moments were measured by indirect ELISA as described by Ploegaert *et al.*[29] Levels of both classical and alternative complement activity in all birds at several moments were determined as described previously.[30]

To determine the effects of different feeds on specific cellular reactivity *in situ*, a lymphocyte stimulation test (LST) was used with concanavalin A for T-cell and LPS for B-cell stimulation according to earlier described methods.[31] Additionally, lymphocyte proliferation in reaction to feed extracts was measured *in vitro* by lymphocyte stimulation test.

Metabolomics analyses. Metabolomics analyses were performed on plasma obtained before and after the KLH challenge at weeks -1, 1 and 3 and on liver tissue after dissection (week 4). All animals of both C-groups and of a limited number (6) of both H- and L-line groups were analysed by TNO Quality of Life.

The plasma and liver samples were analysed by different analytical methods covering a wide range of classes of metabolites:

GC-MS. Plasma (100 μl) or liver (10 mg) samples were, respectively, deproteinised and extracted with methanol and subsequently derivatised, i.e. oximation and silylation. The derivatised samples are analysed by GC-MS. Classes of metabolites that can be analysed with this method are amino acids, mono- and disaccharides, organic acids, amines, alcohols etc. For more details, see Koek *et al.*[32]

Lipid liquid chromatography-MS. Plasma (10 μl) and liver (5 mg) samples were extracted with iso-propanol. After centrifugation, the supernatant was analysed by reversed-phase liquid chromatography-MS (LC-MS) using a water-MeOH gradient and electrospray ionisation in the positive mode. With this method, various classes of lipids can be analysed, e.g. diglycerides (DG), triglycerides (TAG), cholesteryl esters (ChE), phosphatidylcholines (PC), lysophosphatidylcholines (LPC) and sphingomyelines (SPM). For more details, see Verhoeckx *et al.*[33]

Bile acids/NEFA liquid chromatography-MS. Plasma (50 μl) and liver (5 mg) samples were extracted with methanol. The resulting extract was analysed by reversed-phase LC-MS using a water-MeOH gradient, C18 column and electrospray ionisation in the negative mode. With this method, NEFA and bile acids (BA) can be analysed as well as several unknown metabolites. For more details, see Bobeldijk *et al.*[34]

Samples were analysed using standardised protocols, including randomisation, internal standards and quality control samples. Data pre-processing was carried out by composing target lists for all platforms based on retention time and mass-to-charge ratio (*m/z*) and the peaks of all components were integrated. All peak areas were subsequently normalized using internal standards. These target lists were used for further statistical analysis.

Genomics analyses. Gene expression was analysed in jejuni samples obtained after dissection, using whole genome chicken cDNA arrays. All animals of both C-groups and a limited number (6) of both H- and L-line groups were analysed by Wageningen UR - Central Veterinary Institute, as described by Van Hemert *et al.*[35]

A *post-mortem evaluation* was performed by pathologists of RIKILT on all the animals of both C-groups and on a limited number (6) of both H- and L-line groups. After fixation, the tissues were routinely processed and embedded in paraffin wax. The thymus and bursa were weighed and all gross pathological alterations were described during processing of the tissues. Sections of 4 μm were cut on a microtome (Leica RM 2165) once for each formalin-fixed tissue specimen and dried overnight in a stove at 35°C. The sections were stained with Mayer's haematoxylin and eosin (H&E). For staining T-lymphocytes in the intestines, an immunohistochemical method with anti-CD8 (Rabbit polyclonal AB, Lab Vision Immunologica Duiven, The Netherlands) was used. Moreover, for apoptosis, sections were stained using Apoptag peroxidase in situ apoptosis detection kit (MP Biomedicals, Amsterdam, the Netherlands). Immunohistochemical staining was performed with an automatic immunostainer (Ventana Benchmark, Ventana Medical Systems, Illkirch CEDEX, France). The sections of the duodenum and jejunum were scored for the villus length/crypt length ratio by measuring the length of 3 villi and 3 crypts per section from which the mean value was calculated. Measurements were performed using Leica Quips image analysing system (Leica Image Systems, Cambridge, UK).

Statistical methods

Univariate statistics were computed for measurements on chickens grouped by organic and conventional feed: mean; standard error of the mean (SEM); standard deviation; 95% confidence interval for the mean, median, minimum and maximum. Mean differences between the two feed groups were tested with ANOVA[36], two-sample *t* test with pooled variance[37] and Wilcoxon rank sum test for equal medians.[38]

Metabolomics data were analysed with principal component analysis (PCA) to explore the structure of the variables[175] and their relation with the chicken lines and feed. In metabolomics data, the number of variables was reduced. The best 20 variables enabling to discriminate between the organic and conventional feed groups were selected for each platform using cross-validated linear discriminant analysis[39] on all available time points per variable. The mutual relations between the predictive variables were explored with principle component analyses on standard normalised variables (*z*-scores). To facilitate interpretation with respect to the influence of the two feeds, partial least squares discriminant analysis (PLS-DA)[40,41] was applied (results not shown here). Results are considered significant at a $p<0.05$ level. All data analysis was performed with Matlab software (version 7.3.0 R2006b, The Mathworks, Natick, MA, USA).

Results

Diets

Results of analyses of the feeds are presented in Table 2.
Comparison of the nutritional content of the organic and conventional feeds showed most consistent differences in the amount of proteins and several amino acids, which was about 10% higher in the conventional feed. This was due to higher levels of proteins in conventional wheat, soya and barley (results not shown). Some differences in fat and carbohydrate content between the conventionally and organically produced feeds were found, though differences were lower than 10%.The only exception was the fat content in the grower feed of the second generation, being fed in the period of the KLH challenge, which was 15% higher in the organic feed, with higher levels of unsaturated C18 fatty acids. Differences in fat content of the feed were especially due to the higher amount of fats in organically produced soya. The two feeds were energetically approximately equal as shown by the values for metabolisable energy (ME), while the lower content of protein in the organic feed was energetically compensated by the other macronutrients. Further several, but not always consistent, micronutrial differences were observed (Table 2).
Lower levels of LPS endotoxins were found in organic than conventional starter feeds (11.5 vs 13.9 endotoxin unit (EU)/mg) and grower feeds (14.5 vs 19.0 EU/mg) of the second generation. The counts of gram-negative bacteria, however were found to be higher in organic ingredients of all feeds, although in varying amounts. Within the starter, grower and layer feed groups, the difference in Enterobacteriaceae levels between the organic and conventional production varied a factor1.2-10. The largest difference was found in the first batch of first generation layer feed (5.3×10^4 vs. 5.3×10^3 colony-forming unit/g. In the second generation, the observed levels in the

organic vs. conventional feeds were 2.7 x 10^4 vs. 1.4 x 10^4 colony-forming unit/g in the starter feed and 19.5 x 10^3 vs. 1.8 x 10^3 in the grower feed.

Observations on the animals

General health parameters

First generation. Body weight and growth rate did not differ significantly between the feed groups; the H-line showing the lowest; C-line in between; and the L-line the highest average body weight.

Table 3 displays the weights of the animals of the first generation at 13 weeks of age, which is the age at which the animals of the second generation were sacrificed, as well as the weights at 31 weeks of the first generation, when eggs were gathered for brooding.

Feed intake did not differ between lines in the first generation. Onset of egg laying was slightly earlier among the C-line animals than in the H- and L-line; the animals on the organic feed started egg laying slightly, but not significantly, earlier. Number, weight and quality of the eggs did not differ significantly between the feed groups.

Second generation. All animals of the second generation were diagnosed as being perfectly healthy at 10 weeks by a poultry veterinarian. Feather development was slightly faster in the conventional group, but the difference could not be objectified by feather length measurements.

Feed intake of the second generation is shown in Figure 2. In the second generation, feed intake was measured per run, each run housing a group of 6 animals, with 2 animals of each of the 3 lines. Accordingly, feed intake could not be discriminated between lines.

At week 7 the feed was changed from starter to grower feed. After the change to grower feed, intake of the conventional feed started to increase, being significantly higher than the intake of organic feed in week 10, 12 and 13. After 13 weeks animals fed conventional feed cumulatively consumed about 80 grams more feed compared to animals on organic feed (3686 g vs. 3607 g).

Body weight of the second generation of all lines is shown in Figure 3. A difference in body weight, related to the diet treatments appeared, the body weight of the C-line animals on conventional feed being significantly higher than the body weight of the animals on organic feed, and the difference increasing during lifespan. Table 3 shows the weights of the animals of the second generation at 13 weeks, in comparison to the first generation. At 13 weeks the conventional C-line animals weighed significantly more than the organic C-line animals, the difference being larger than in the first generation. In the H- and L-line hardly any diet effects on body weight were found. H-line animals on organic feed were significant heavier only in the first week. L-line animals on the conventional feed were heavier during the first three weeks, after that there was no significant difference (Figure3).

Table 2 Analyses of the starter, grower and layer feeds of conventional and organic origin of the first and second peer group

		First generation						Second generation			
		Grower		Layer-first batch		Layer-second batch		Starter		Grower	
Nutrient	**unit**	**Conv.**	**Org.**	**Conv.**	**Org.**	**Conv. †**	**Org. †**	**Conv.**	**Org.**	**Conv.**	**Org.**
Metabolisable Energy	Kj/kg	14610	14480	14302	14487	14061	14059	14882	14729	15102	15231
Carbohydrates total	g/kg	539	546	538	555	559	562	620	624	574	585
Crude fat (acid hydrolysis)	g/kg	59	61	64	69	46	51*	42	42	53	62*
Protein	g/kg	192*	173	164*	147	168	154	164	151	199*	176
Raw fibre	g/kg	42	43	40*	36	42*	37	34	36	39	39
Moisture content	g/kg	120	126	120	116	122	117	123	119	120	122
Ash content	g/kg	48	51	114	113	105	116	51	64**	54	55
Fatty acids											
C16:1 c9	total fat%					0.1	0.1	0.1	0.1	0.1	0.1
C18:0	total fat%					4.0*	3.6	3.2	3.2	4.0*	3.5
C18:1 c	total fat%					21.9	24.2*	21.3	23.4	23.2	23.2
C18:2 c9.12	total fat%					50.9	50.7	50.9	47.3	48.4	51.3
C18:3 C9.12.15	total fat%					5.8	5.7	5.1*	4.5	5.1	5.9*
Amino acids											
cystine	g/100 g	0.34	0.33	0.29	0.28	0.29	0.30	0.33	0.30	0.33	0.32
methionine	g/100 g	0.29*	0.26	0.28	0.26	0.40	0.49*	0.39	0.45*	0.30	0.29
aspartic acid	g/100 g	1.87*	1.66	1.63	1.49	1.52	1.52	1.79*	1.52	1.80	1.80
threonine	g/100 g	0.72*	0.65	0.64	0.62	0.62	0.63	0.73	0.68	0.72	0.68
serine	g/100 g	0.95*	0.84	0.82	0.75	0.80	0.78	0.89*	0.79	0.96	0.88
glutamic acid	g/100 g	3.88*	3.3	3.27*	2.83	3.19	2.96	3.30*	2.97	3.90*	3.40
proline	g/100 g	1.23*	1.07	1.01	0.94	1.02	0.96	1.23*	1.04	1.20	1.10
glycine	g/100 g	0.81*	0.71	0.72	0.66	0.69	0.68	0.79*	0.72	0.81	0.75
alanine	g/100 g	0.86*	0.78	0.77	0.72	0.75	0.76	0.83	0.76	0.86	0.82

valine	g/100 g	0.91*	0.8	0.81	0.76	0.78	0.77	0.94*	0.83	0.91	0.85
isoleucine	g/100 g	0.83*	0.73	0.71	0.67	0.70	0.68	0.78	0.72	0.81	0.75
leucine	g/100 g	1.54*	1.36	1.37	1.3	1.35	1.32	1.52	1.41	1.50	1.40
tyrosine	g/100 g	0.7*	0.62	0.65	0.61	0.61	0.58	0.71*	0.63	0.68*	0.62
phenylalanine	g/100 g	0.95*	0.82	0.87*	0.77	0.81	0.78	0.95*	0.84	0.95*	0.86
histidine	g/100 g	0.49*	0.44	0.45*	0.4	0.42	0.39	0.42*	0.37	0.47	0.44
lysine	g/100 g	1.02	0.94	0.91	0.87	0.88	0.88	1.02*	0.89	1.00	0.98
arginine	g/100 g	1.39*	1.21	1.12	1.04	1.15	1.08	1.10	1.01	1.40*	1.20
tryptophan	g/100 g	0.24	0.23	0.22*	0.19	0.20	0.19	0.23*	0.2	0.23*	0.20
Ratio protein/crude fat		3.25*	2.84	2.56**	2.13	3.65**	3.02	3.90	3.60	3.75**	2.84
Chloride	g/kg	2*	1.7	1.4	1.3	2.1**	1.7	2.1	2.3	2.3**	1.9
Alfa-tocopherol	mg/kg	14	16*	13	16*	15	14	15	16	13	13
Beta-tocopherol	mg/kg	3.7	3.7	2	2	2.7	2.6	2.7	2.7	2.8	3.1*
Gamma-tocopherol	mg/kg	37	43*	32*	27	47**	38	10	11	25	27
Delta-tocopherol	mg/kg	11	18**	11	12	15	14	2.1*	1.9	9.3	14**
Total folate	mg/kg	0.5	0.7**	0.5	0.5	0.6	0.7*	0.3	0.4**	0.6	0.6
Trace elements											
Se	ug/kg	290*	260	290	270	290	330*	290	330*	280	270
Fe	mg/kg	250	240	260	280	220	340**	220	260*	330**	240
Cr	mg/kg	0.7*	0.6	3.1	3.9**	2.9	5.2**	1.3	1.4	1.5*	1.3
Mn	mg/kg	82	80	110	130*	92	110*	130	150*	110*	100
I	ug/kg	1200	1300	700	750	780	930*	870	940	940	1000
Cd	ug/kg	39	45*								
Pb	ug/kg	170**	110	200	250**	190	240**	98	130**	190**	140
As	ug/kg	120**	70	120	130	110	150**	110*	100	110**	64

† analyses were performed after the feed was supplemented with some essential amino acids up to the required level.
*≥10% higher than the conventional or organic feed; ** ≥20% higher than the conventional or organic feed.

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Table 3 Body weight of first and second generation high, control and low chicken line (H-, C-, L-lines) animals on organic or conventional feed at 13 weeks for both generations and at 31 weeks of the first generation (Mean values and standard deviations)

	H-line				C-line				L-line			
	Org.		Conv.		Org.		Conv.		Org.		Conv.	
Body weight	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
1st generation												
Age 13 weeks (g)	1050	30	1033	30	1183	18	1205	28	1241	28	1185	25
Age 31 weeks (g)	1882	64	1905	49	1971	38	1995	46	2154	62	2167	43
2nd generation												
Age 13 weeks (g)	1048	18	1050	15	1098	24	1241	27	1209	21	1209	12

Org., organic; Conv., conventional.

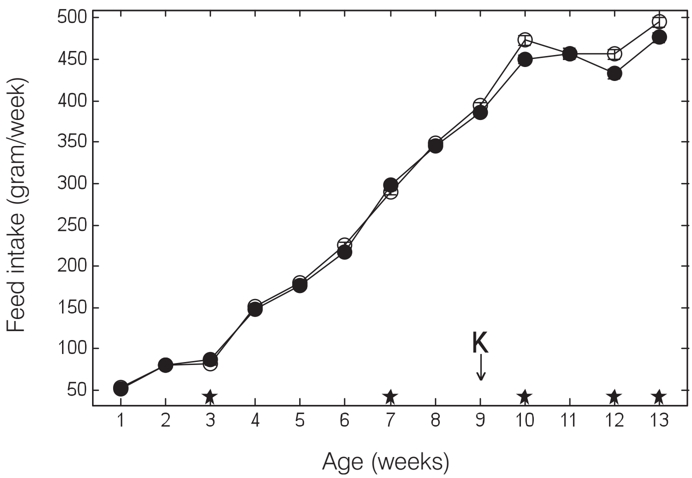

Figure 2 Feed intake per week. Second generation H-,C- and L-line together. (●), Organic feed; (○), Conventional feed. K=KLH challenge. Values are means (n=13 runs of 6 animals), with standard errors of the mean represented by vertical bars, significant differences indicated by stars ($p<0.05$).

Growth rate of the second generation of all three lines is shown in Figure 4. After the challenge growth rates differed significantly between the diet treatments in all lines. The animals of the C-line on conventional feed were significantly ahead compared to the animals on organic feed till week 8. After the challenge, which took place in week 9,

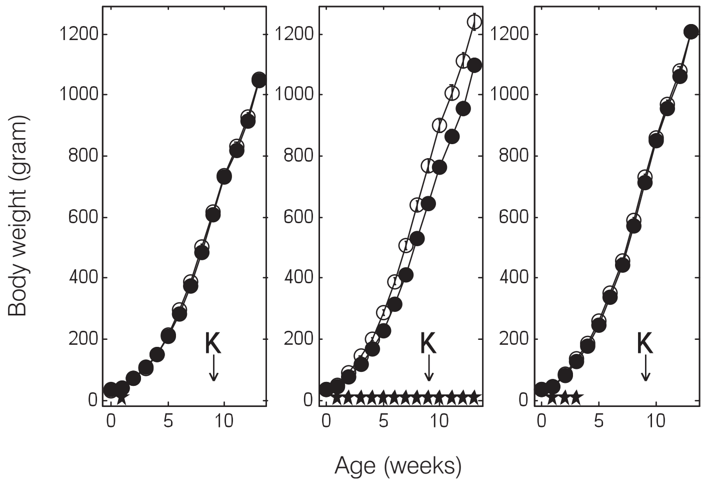

Figure 3 Body weight. Second generation H-line, C-line and L-line. (●), Organic feed; (○), Conventional feed. K=KLH challenge. Values are means (n=22-26) with standard errors of the mean represented by vertical bars, significant differences indicated by stars ($p<0.05$).

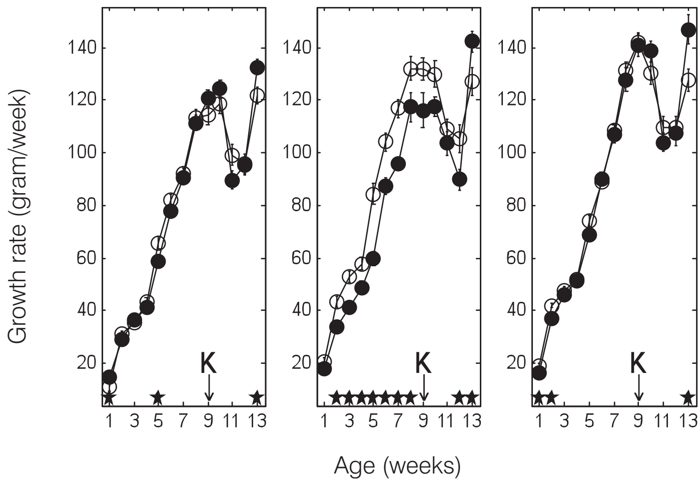

Figure 4 Growth rate of body weight. Second generation H-line, C-line and L-line. (●), Organic feed; (○), Conventional feed. K=KLH challenge. Values are means (n=22-26) with standard errors of the mean represented by vertical bars, significant differences indicated by stars ($p<0.05$).

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growth started to decline in week 10 in both feed groups for a period of 2 weeks, which was significantly stronger in the organic group than in the conventional group in week 12. From week 12 onwards, the growth of the organic animals showed an acceleration and overtook significantly the growth rate of the conventional animals, till life termination at week 13.3.

Growth of H- and L-line animals was similar between the feed groups till week 12, also in the decline of growth after the challenge. In week 13, both the H- and L-line animals on organic feed showed a stronger 'catch-up growth', leaving the animals on conventional feed behind.

Immune parameters

Clear differences were observed between the different line-feed groups, which were related to the different chicken lines, the different feeds and the changes in feed.

With regard to the first generation it is worth mentioning that production of AB levels to the NCD vaccine, representing the specific component of the immune system, was significantly increased in animals on organic feed of the L-line, up to a level comparable of the H-line birds (Figure 5).

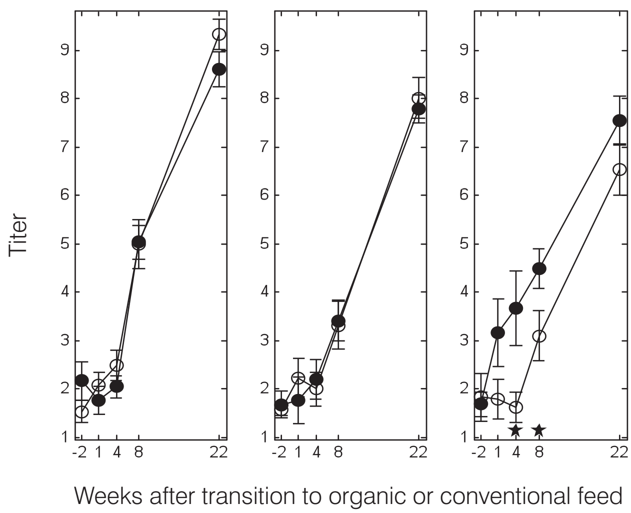

Figure 5 NCD-specific antibody titres. First generation H-line, C-line and L-line. (●), Organic feed; (○), conventional feed. Values are means (n=11-13) with standard errors of the mean represented by vertical bars, significant differences indicated by stars ($p<0.05$).

In the second generation, titres of natural AB binding to LPS, representing an innate component of the immune system, showed a strong rise in the weeks following the KLH challenge in all groups of animals. It was regardless of the type of feed highest in the H-line, followed by the C-line and lowest in the L-line (Figure 6).

The C-line showed a differential response wherein the animals on organic feed showed the highest response at 2 weeks after the challenge followed by a gradual decrease, while the animals on conventional feed showed a response that was still increasing at week 4. The (control corrected) LPS-stimulated monocyte reactivity, representing a cellular of the innate immune system, was significantly higher in the organic animals of the H- and C-lines 1 week before the challenge, which was 1 week after the change from starter to grower feed (Figure 7).

The KLH-specific AB titres, reflecting the specific immune system, showed a strong rise in all lines after the KLH-challenge, but did not show differences between the feed groups (data not shown). However, the AB levels on the NCD vaccine showed an effect of the KLH challenge by an increase in titres, divergent in height for the different lines (Figure 8).

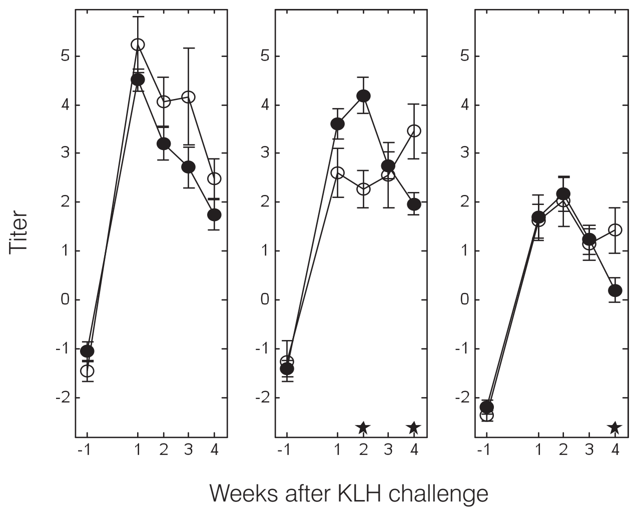

Figure 6 Lipopolysaccharide binding natural antibodies. Second generation H-line, C-line and L-line. (●), Organic feed; (○), conventional feed. Values are means (n=21-26) with standard errors of the mean represented by vertical bars, significant differences indicated by stars ($p<0.05$).

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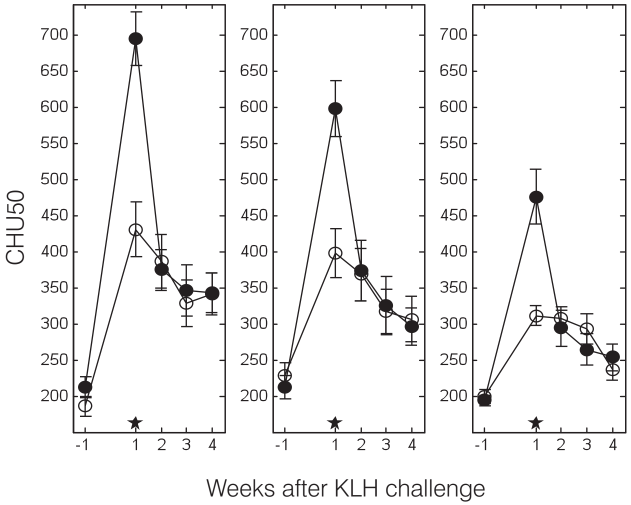

Figure 7 Control corrected lipopolysaccharide-stimulated NO production in monocytes. Second generation H-line, C-line and L-line. (●), Organic feed; (○), conventional feed. Values are means (n=16-26) with standard errors of the mean represented by vertical bars, significant differences indicated by stars ($p<0.05$).

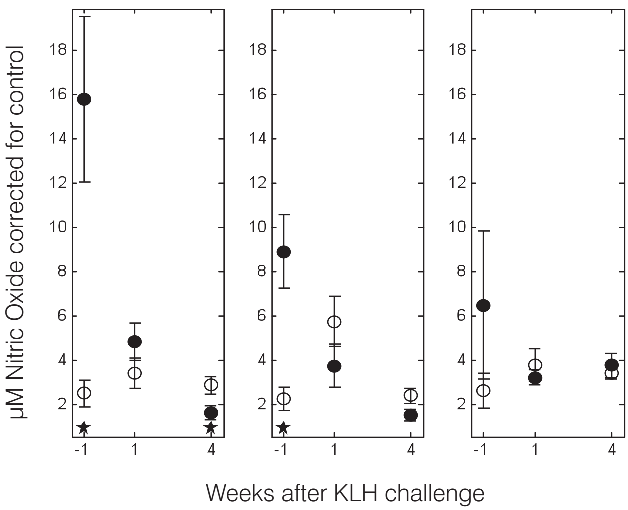

Figure 8 Newcastle disease-specific antibody titres in serum. Second generation H-line, C-line and L-line. (●), Organic feed; (○), conventional feed. Values are means (n=21-26) with standard errors of the mean represented by vertical bars, significant differences indicated by stars ($p<0.05$).

The response of the animals on the organic feed showed a peak, stronger than that of the animals on conventional feed, at week 2 (H- and L-Line) or 3 (C-line), followed by a decrease. The reactivity of the classical complement system was significantly stronger in all organic animals at 1 week after the challenge (Figure 9).

In the lymphocyte stimulation test in whole blood, representing the cellular component of the specific immune system, the B-lymphocyte response was significantly higher, and the T-lymphocyte response was higher but not significant, of the organic animals in the week after the KLH-challenge (data not shown). In the lymphocyte stimulation assay *ex vivo*, addition of feed extracts of the organic and conventional feed to Con A stimulated whole blood cell cultures at three weeks after the challenge did result in significantly more proliferation of T-cells of animals on the organic feed compared to animals on the conventional feed (data not shown).

Metabolomics

Plasma. All three analytical platforms showed significant differences in metabolite concentrations between the organic and conventional groups, whereas only the GC-MS method allowed for a clear separation of the animals according to their

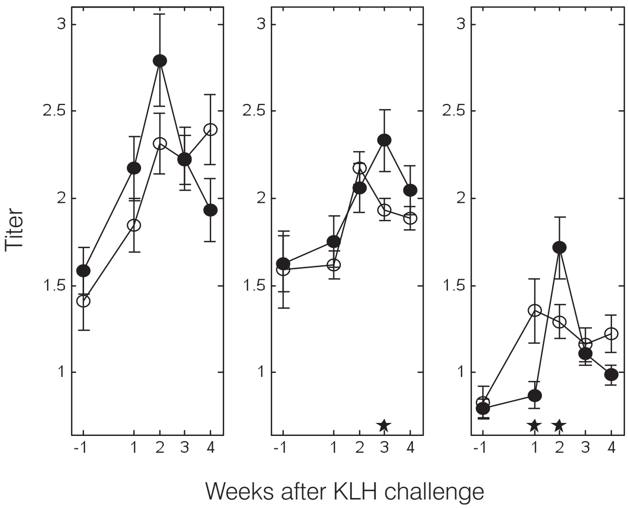

Figure 9 CH50 activity of serum after classical complement activation. Second generation H-line, C-line and L-line. (•), Organic feed; (o), conventional feed; CH50, haemolytic complement (units per ml), the dilution of serum required to lyse 50% of the erythrocytes in the essay; KLH, keyhole limpet haemocyanin. Values are means (n=22-26) with standard errors of the mean represented by vertical bars; significant differences indicated by stars ($p<0.05$).

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genetic background. The lipid LC-MS method showed the most strong treatment differences in the discriminant analysis (Figure 10).

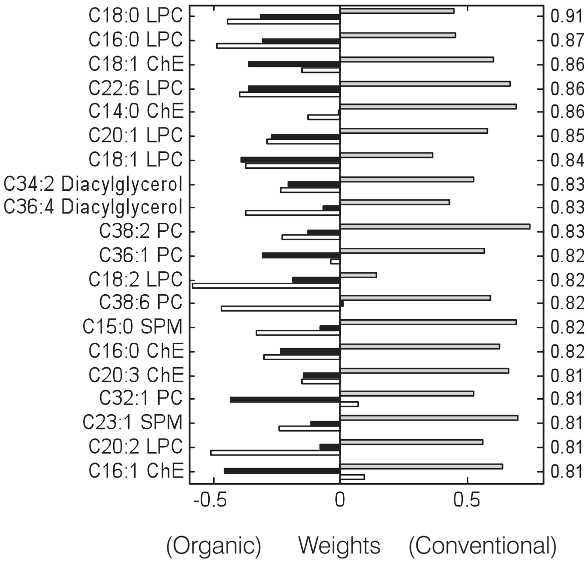

Figure 10 2nd generation C-line. Plasma lipid LCMS discriminant weights of discriminant function.

Discriminant analysis for treatment on Lipid platform (n=42). The grey bars show the discriminant weights on time point -1, the black bars on time point +1 and the white bars on time point +3. On the right hand side, the cross-validated rate of correct classification is shown for each metabolite.

A general pattern could be observed for the plasma lipids, where the organic group showed significant lower lipid concentrations compared to the conventional group 1 week before the challenge, whereas higher concentrations at the time points after the challenge (weeks 1 and 3). Notably, 7 out of 11 plasma lysophosphatidylcholines (LPC) measured were identified as highly discriminating metabolites between treatments. The LPC with the largest differences between the feed groups were LPC18:0 and LPC16:0. Their response pattern was comparable in the different chicken line-feed groups: LPC in the organic group had highest concentrations after the challenge, whereas LPC levels in the conventional group decreased after the challenge. Other compounds that strongly discriminated between the two treatments were phosphatidylcholines (PC) and cholesterol esters (ChE). The general pattern of response was identical to the pattern observed for the LPC.

In the discriminant analysis of the bile acids/NEFA LC-method (Figure 11), a general pattern could be observed for the plasma NEFA. In general, NEFA showed similar concentrations in the two feed groups one week before the challenge, but were significant higher in the organic group compared to the conventional group in the weeks after the challenge with highest levels at t = 1. The NEFA C22:6, C18:0, C18:1 and C20:3 were found as most discriminating between the organic and conventional chicken lines. Only two bile acids (taurocholic acid (TA) and cholic acid (CA)) out of 15 measured were found amongst the top 20 discriminating metabolites, having a lower discriminating power than most NEFA.

The metabolites from the GC-MS method (Figure 12) allowed a relatively clear separation between the different chicken lines, as well as according to their treatment. Upon closer inspection of the partial least squares models, the metabolites lysine, glycerol and α-ketoglutaric acid were most related to line differences, while glycerol and the NEFA C16:1, C18:1 and C18:2 were most related to treatment differences.

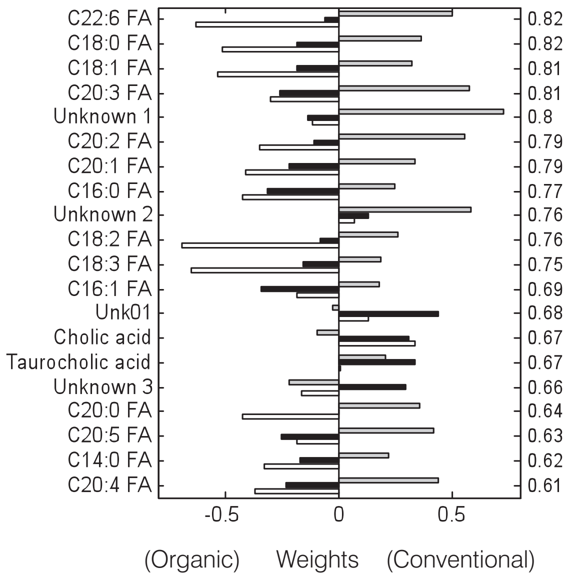

Figure 11 2nd generation C-line. Plasma bile acid / FFA discriminant weights of discriminant function.

Discriminant analysis for treatment on Lipid platform (n=41). The grey bars show the discriminant weights on time point -1, the black bars on time point +1 and the white bars on time point +3. On the right hand side, the cross-validated rate of correct classification is shown for each metabolite.

Glycerol and the NEFA of the organically fed animals had significant higher concentrations at the time points after the challenge compared to the conventional animals.

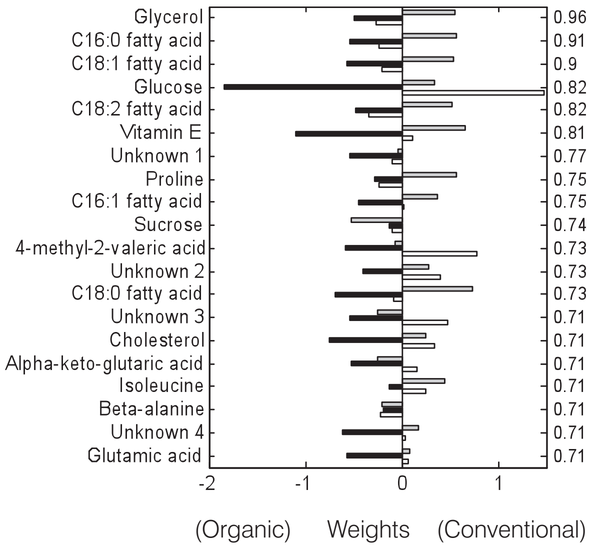

Figure 12 2nd generation C-line. Plasma GCMS discriminant weights of discriminant function.

Discriminant analysis for treatment on Lipid platform (n=42). The grey bars show the discriminant weights on time point -1, the black bars on time point +1 and the white bars on time point +3. On the right hand side, the cross-validated rate of correct classification is shown for each metabolite.

Liver Only metabolites analysed with the GC-MS platform showed significant concentration differences between the treatments in liver samples. None of the metabolites analysed with the three metabolic platforms allowed for a significant discrimination between the genetic backgrounds of the animals. Liver samples from the organic animals had higher concentrations of glyceric acid, alanine, monomethyl phosphate and the sugars ribose, ribulose and fructose compared to the conventional animals. Furthermore, the amino acids alanine and methionine, vitamin E and ńketoglutaric acid were increased in livers of the organic animals.

Genomics

After analysis thirty genes were found to be differentially regulated between the two feed groups, independent of their genetic background. Of these thirty genes, seven were involved in cholesterol biosynthesis. Genes involved in cholesterol biosynthesis were upregulated in jejuni from the organically fed animals. Other genes found to be regulated were involved in immunological processes, like B-G protein (part of chicken myosin heavy chain), chemokine ah221, and the immunoglobulin heavy chain. Microarray data were confirmed using qPCR analysis.[43]

Post mortem results

The veterinarian dissecting the animals did not perceive abnormalities, but observed some more fat tissue in the animals on conventional feed. In general, the histological observations showed a variation that normally can be expected in the animals. In the C-line animals, the relative weight of the bursa was significantly higher in the animals on organic feed. In the H-line, the relative gastrointestinal weight was higher in the organic animals. Some animals on the conventional feed showed fat deposits in the submucosa of the caecum.

Discussion

The present study investigated effects of two identically composed feeds from different agricultural production systems, using a chicken model for future studies in human subjects. It focussed on differences caused by feeds, whereas all other factors that normally differ between organic and conventional husbandry systems such as housing, space per animal, temperature changes through outdoor allowance, etc. were kept the same. The present study showed differences in body weight, growth rate, immune, physiological, metabolic and gene regulation parameter.

The present study is the first largest study in animals investigating the effect of feeds from different agricultural systems, using animals from different immunological lines. The study contributes to the discussion on the effect of organic food on health, because of its large number of animals (n=150) and the use of three selection lines over two generations. 9

In the present study the choice was made to collect ingredients from neighbouring organic and conventional farms. Both organic and conventional farming systems have their varieties suitable for their specific agricultural systems, so we accepted such difference in varieties. This was the case for wheat, barley and triticale. Some authors argue that comparisons between organic and conventional products should be solely performed with products from controlled field trials, using the same varieties.[44] However, such trials never represent the complete agricultural system and it can be disputed how representative the use of the same varieties is. Other authors describe the need of different varieties, specifically adapted with their root systems to the different fertilization practices in either organic or conventional agricultural systems.[45,46,47,48,49] We considered choosing 'best practice farm pairs', representing the full systems, as the best alternative.

It is well known that within organic produce, there is, like in conventional produce, a large variation in product quality.[4] Therefore it cannot be excluded that similar differences would have been found if different batches within one cultivation system were compared, rather than the present comparison of two cultivation systems.

The aim was to identify biomarkers for future human intervention studies. The results of the present study cannot be directly extrapolated to humans. Chickens do not have differentiated, organised lymph nodes like mammalians have, but the avian immune system is besides that comparable to the mammalian immune system. It is the immune system of chicken that has provided most basic insights in immunology.[50] Because of the availability of selected chicken lines, interactions between genetic background and feed could also be found. The high line consisted of high immune responders, the control line represented a random selection of the average chicken population, and the low liners generally show much lower immune responses.

By choosing a two-generation design, we could limit the effect of maternal or paternal conventional feed intake. The main group of interest was the second generation of chickens. By including also immunological analyses of the first generation, we could compare effects from first time exposure of the animals to the experimental feeds, as occurred in the first generation, with effects of secondary exposure to the feeds by the chicks of the second generation. These animals may already have been epigenetically influenced by the nutritional status of their parents. It was clear that the high and low responder groups reacted overall differently to similar feed intakes than the control line. For instance, for body weight hardly an effect of the different types of feed could be observed in the two extreme lines, although they showed the previously described differences in body weight, relating to the different AB responses.[18] It seems that their genetic constitution prevented them in reacting flexible to changing (phenotypical) influences, whereas the control line displayed the ability to react to feed intake. Only in the last week of the experiment, in response to the challenge, a discriminating effect between the feed groups was seen in the growth curves of both the H- and L-lines.

All animals were healthy, as both feeds were sufficient nutritious. Though differences between the conventional and organic feeds were not large besides a 10% higher content of protein in the conventional feed, and the amount of metabolisable energy was comparable, the present study showed differences between feed groups in body weight and growth rate, in several immune parameters and in genomics and metabolomics measurements.

Body weights differed significantly for the C-line animals, the conventionally fed group being heavier during the whole period. Several factors might have influenced the body weight of the animals, of which energy intake and the available amount of amino acids are most influential. Of these the sulphur containing amino acids methionine, cysteine and lysine are most essential.[51,52] The organically fed animals ate more in weeks 3 and 7, while the conventionally fed animals ate more in weeks 10, 12 and 13. As described, the etabolisable energy was approximately the same, but the protein content and several amino acids in the conventional diet were 10% higher. The bioavailability of amino acids was evaluated in the starter feed, and among the amino acids, there were no limiting factors.

The difference in feed intake and/or the difference in protein content might explain differences in growth. In the Lauridsen rat study[15], however, the rats on a diet from production with high fertilisation with pesticides (comparable to conventional) displayed more fat tissue and (NS) higher body weights, though feeds were made isoenergetic, as well as supplemented till equal levels of protein. These results could not be explained.

Concerning the immune system, in the first generation, the L-line animals on organic feed displayed a significant increase in NCD vaccine-specific AB titres. In this group of animals with a lower immune responsiveness, this is a remarkable effect in the specific immune system. In the second generation the animals on organic feed appeared to have a stronger immune competence in the innate immune system than the animals on conventional feed, mostly in the C-line, but also several times in the H- and L-line, reflected by the enhanced responses of monocytes before the challenge, 1 week after a feed change from starter to grower feed, and by the higher levels of LPS-binding natural AB titres after the challenge. The challenge activated the specific immune system stronger, reflected by the increased NCD-specific vaccination titres and the activation of the classical complement route after the challenge. KLH-specific titres were high after the challenge in all animals but not discriminating between the feed groups. To our opinion, this does not reject our proof of principle that the organic feed is immune modulating. We think it might be too simplistic to expect uniform effects in all parameters. Though specific isotypes were not distinguished, it is not unlikely that the enhanced NCD vaccine titres may rest on enhanced IgG levels, comparable to the Lauridsen study.

Plasma metabolomics analysis showed a distinct metabolic pattern after the KLH challenge, which was in accordance with the immunological findings. Especially, intermediates of lipid metabolism showed most pronounced differences in the organic group. Increased concentrations of LPC, NEFA and glycerol could be recognized as remains of an acute phase reaction (APR; Khovidhunkit *et al.*[53]). An APR, caused by injection of an immunogenic protein, increases TAG metabolism and fatty acid turnover, resulting in increased plasma levels of NEFA and glycerol.[53]

Increased levels of plasma LPC and saturated LPC can be caused by a transient increased activity of secretory phospholipase A2[54] in APR. In the lipid platform, increased amounts of cholesterol esters and phosphatidylcholines were found, probably related to APR.[53]

The increased gene activity of the cholesterol pathway as found in the jejunum of the organic animals by genomics analyses[43] can, however, not be explained by this APR, as that reaction occurred soon after the challenge, 4 weeks before the sampling of the jejunum.

The different parameters indicate a higher immune modulation by the feeds and tendencies towards an enhanced immune responsiveness or immune competence of the animals on organic feed. In the first generation, the effect is mainly found in the specific immune system, especially in the low line animals, while, in the second generation, it appears mainly in the innate immune system, but also in the specific part. It becomes increasingly clear that innate and specific immune responses are intimately interconnected.[55] The first generation animals received the experimental diets at an age that the innate immune system has been repeatedly challenged. Whether enhanced sensitivity of the innate system of the second generation animals that received the diets early after hatch is based on a dietary challenge of a naïve immune system remains to be established,

The cause of the enhanced immune responsiveness in the organic animals before, but especially appearing after the challenge, is unclear. The organic feed contained more immune-stimulating gram negative bacteria, but at the same time lower levels of immune-stimulating LPS were measured, a contradiction we have no explanation for. The organic feed contained more moulds, which might bring immune stimulating ńglucans, but these were not measured. It is questionable whether all effects can be explained by the differences in bacterial load, as differences between feeds were limited and the quantitative additions through the feeds were relatively small compared to the bacterial load already present in the gut system of the animals.

A lower body weight (gain) in chicken has been related with an enhanced status of the immune system.[18,29,56,57] In the present study, the weight development of the organic C-line animals was comparable to that of the H-line, whereas in the conventional C-line it was comparable to the L-line animals, which suggests that the lower body weight gain in organically fed birds could be related with an enhanced immune reactivity.

The question remains how enhanced (immune) reactivity to the immune challenge of the animals fed organic feed should be evaluated from a perspective of health. A baseline reaction on KLH in chicken is not known. In mice, Demas *et al.*[58] described the effect of KLH, as provoking an increased metabolism, increased body temperature (fever among several animals), accompanied by body catabolism connected to anorexia. Demas *et al.* interprets this process as energy costs of mounting an immune response. The observed symptoms in our animals reflect the ones described by Demas *et al.* Body temperature was not measured, but parameters in the metabolomics as well as in the immunological analyses indicated an APR, most strongly in the animals fed organic feed. An APR is not always considered positive as it can be devastating for the organism.[59] In the present study, the growth rate is indicative for a positive performance of the animals after an APR. After the depression of growth, in all animals during 2 weeks after the challenge, animals on both diets started to grow again, first those on conventional feed, then the animals on the organic feed showed an increased catch-up growth, which appeared in all three lines. It might reflect the activated pentose phosphate pathway in the liver recognized by the increased concentrations of the sugar metabolites ribose, ribulose and fructose in the livers of the organic animals. In human medical literature, catch-up growth is taken as a clinical sign of recovery after illness for children described by Adamkin[60] and Rivkees.[61]

Future studies should follow growth patterns after a challenge during a longer period that the present study did, to be able to evaluate the performance of the organism.

The aim of the present study was to identify biomarkers that indicate potential health effects from organic food. These were identified in various different parameters such as feed intake, body weight and growth rate, in immunological, physiological and metabolic parameters, several of these differing most pronounced after the challenge. The genomics results are remarkable but cannot be connected to the other results yet. The results of the present study showed a tendency, which is slightly similar to some results from previous animal studies with organic feed, although these were quite different in study design. Lauridsen *et al.*[15] found more body fat in rats on a conventional diet and higher IgG AB titres in the animals on an organic diet. Finamore *et al.*[14] found a higher responsiveness of the lymphocytes in rats fed organic wheat, than in animals fed conventional wheat, when the animals were malnourished. Finally, Kummeling *et al.*[62] found in a prospective study in children a reduced risk for eczema at 2 years of age, associated with the consumption of organic dairy. From the present data, we conclude that diets from different origin, i.e. organic vs. conventional production systems, can induce physiological changes in two generations of chickens. Further studies should establish these findings and should unravel the mechanisms underlying our observations.

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The authors state that there are no conflicts of interest.

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Chapter 10

Effect of organically and conventionally produced diets on jejunal gene expression in chickens

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Abstract

Using a nutrigenomics approach we studied the response of second-generation chickens at a transcriptional level to organically grown feed ingredients compared to conventionally grown feed ingredients. Both diets consisted of the same amounts of ingredients, the only difference was the production method. Gene expression was analysed in jejuni using whole genome chicken cDNA arrays. After analysis, forty-nine genes were found to be differentially regulated between chickens fed on the different diets, independent of their genetic background. Of these forty-nine genes, seven genes were involved in cholesterol biosynthesis. Genes involved in cholesterol biosynthesis were higher expressed in jejuni from organically fed birds. Other genes found to be regulated were involved in immunological processes, like B-G protein (part of chicken major histocompatibility complex), chemokine ah221, and the immunoglobulin heavy chain. Using quantitative PCR the effect of genetic background on the differential expression of genes was studied. Differences in gene expression existed between animals fed different diets as well as between different chicken lines. This indicated that diet and genetic background influence the transcriptional response of the jejunum. This is the first time that significant differences in gene expression were shown between animals on diets with organically or conventionally produced ingredients.

Keywords: Organic feed; diet; nutrigenomics; chickens

Introduction

Organic food production is characterised by the absence or limited use of synthetic herbicides, pesticides, insecticides, growth regulators and livestock feed additives. Besides, only organic fertilisers are used such as animal or green manure, and long crop rotation is applied[1,2] In recent years, many consumers turned to organic foods, expecting organic products to be healthier than conventionally grown products. The composition of organically grown ingredients has been studied extensively[3,4], however studies on the effects of consumption of organically dietary components are limited and have not led to conclusive results concerning (beneficial) effects on health.

Nowadays, it is generally accepted that food consumption impacts gene expression, metabolome and finally human health.[5] Recently, several publications described a relationship between diet (components) and gene expression that relates to difference in disease incidence. E.g. the low incidence of certain cancers in the Mediterranean area was suggested to be caused by the Mediterranean diet.[6] Menendez *et al.*[7] showed that olive oil, one of the main ingredients of the Mediterranean diet, inhibits the expression of the HER2 oncogene. Inhibition of this gene does not only exert protective effects against the risk of breast cancer, but can also protect against further progression of disease. Another study demonstrated that rats fed on fresh broccoli for 1 month, showed improved ventricular function of the heart and reduced myocardial infarct size.[8] Broccoli appeared to rescue the cardiomyocytes through regulation of gene expression that led to activation of the survival pathway. Due to novel techniques like genomics, metabolomics and proteomics the molecular responses to diets or dietary components can be studied on a whole genome level, thereby providing insight in the complex interplay of diet and physiology.[9] Summarised, literature describes that diet (components) influences disease status and physiology. However, the gene expression responsible for those influences are mainly unknown. In the present study we describe a nutrigenomics approach to see whether second generation chickens respond differently at a transcriptional level to identically composed diets, from either certified organically or conventionally grown feed ingredients. Gene expression is studied in the jejunum, since the gut is the first contact of diet (components) with the host, and the gut strongly influences the general condition of the host. It is shown that maternal diet influences gene expression in the intestine of offspring, suggesting a role for epigenetic modification of the DNA.[10] Hollingshead *et al.* recently showed that a gestational diet indeed influenced allergic airway inflammation through epigenetic programming[11], confirming that diet can influence the immune status of both mother and child. Therefore, it was decided to feed two generations of chickens to maximise the effects. Mother hens and chickens were fed the described diets, adapted to their age. Gene expression was studied in jejunum of second generation chickens. The described study was part of a larger study, where different physiological parameters were measured and the feed was extensively analysed.[12]. An overview of the experimental set-up and results is given in this issue (Huber *et al.*, Br J Nutr (2010),103:663-676). In our experiment forty-nine genes were found to be differentially expressed between the different diet groups. Several of these genes were involved in cholesterol biosynthesis. Using qPCR the effect of genetic background on the differential expression of genes was studied. In summary, we show evidence that a diet from organically grown feed ingredients induce different genes in chicken jejunum tissue compared to a diet from conventionally grown feed ingredients. To our knowledge this is the first time that clear differences in gene expression are shown due to organically grown feed ingredients.

Materials and Methods

Animal experiment

ISA Brown Warren medium heavy layer hens were divergently selected for 25 generations on their primary antibody response to the multiantigen sheep red blood cells (SRBC) at 5 days after immunisation at 35 days of age. Two selection chicken lines were established: chickens with a high antibody response (H) and chickens with a low antibody response (L). Besides a control line of randomly bred chickens was included (C) resembling the parental stock of origin[13]. The first experimental generation consisted of 71 hens and 22 roosters that were housed in groups until 8 weeks of age, after which they were housed individually. Until 11 weeks chickens were fed normal commercial feed. From 11 weeks the chickens were fed *ad libitum* either organically grown chickenfeed, or conventionally grown chickenfeed. Both feeds consisted of wheat, barley, triticale, peas, maize and soya from neighbouring farm pairs of conventional and certified organic farms with the same basic soil and climatic conditions and preferably the same variety of produce. Feed composition for the different age groups is summarised in Table 1. Via artificial insemination, the second generation was raised. Six groups of 26 second generation chickens were formed: 52 H chickens, 52 L chickens and 52 C chickens that were fed ad libitum either organically or conventionally grown feed according to Table 1.

In contrast to the first generation, the second generation was housed in groups of 6 animals, 2 hens from each line. The runs were spacey and enriched to secure optimal natural behaviour and physiological reactions of animals. The immune system of second generation chickens was triggered by injecting KLH (Keyhole Limpet Hemocyanin), at week 9. Animals were killed at week 13 by cervical dislocation.

Table 1 Composition of chickenfeed

Ingredient	Starter diet (0-6 weeks)	Grower diet (7-17 weeks)	Layer diet (from 18 weeks)
Maize	20 %	20 %	25 %
Wheat	30 %	26.42 %	25.23 %
Barley	5 %	10 %	5 %
Triticale	12.05 %	0 %	0 %
Soyabeans heated	0 %	10.17 %	19.87 %
Soya flakes	10.16 %	20 %	0 %
Peas	10 %	10 %	10 %
Potato proteins	7 %	0 %	2.5 %
MonoCalcFos	1.13 %	0.73 %	1.01 %
FX Layers Premix	1 %	1 %	1 %
Fat of plant origin	1.5 %	0 %	0.52 %
Salt	0.07 %	0.09 %	0.06 %
Chalk	1.64 %	1.16 %	7.65 %
Shells broken	0 %	0 %	2 %
$NaCO_{3\_}$	0.09 %	0.08 %	0 %
Methionine	0.11 %	0.04 %	0.15 %
Total	**99.75 %**	**99.69 %**	**99.99 %**

Tissue samples from several organs were taken and snap-frozen in liquid nitrogen. The animal experiment was approved by the ethical committee of Wageningen University, Wageningen, The Netherlands, in accordance with the Dutch law on animal experiments.

RNA Isolation

RNA was isolated from the jejunum of individual chickens using the Trizol method as described by Van Hemert *et al.*[14]. Tissue was ground under liquid nitrogen in a pestle and mortar. A small volume of ground tissue was dissolved in 1 ml of Trizol (Invitrogen, Carlsbad, CA, USA), and homogenised. The RNA was extracted after addition of 1/5 volume of chloroform. Subsequently, the RNA was precipitated with isopropanol, washed and dissolved in DEPC-water. RNA-concentration and -quality was determined using the Nanodrop (Thermo Scientific, Waltham, MA, USA), as well as by gel electrophoresis. 10 μg of RNA from 4 - 6 individual chickens of the same line were pooled, after which RNA-quality and -quantity was checked again.

Hybridisation of microarrays

A quantity of 5 μg of each pooled RNA sample was labelled and hybridised using the Micromax TSA Labelling And Detection Kit (Perkin Elmer, Wellesley, MA, USA) according to the instructions of the manufacturer with modifications as described by Van Hemert *et al.*[14] On each slide pooled RNA from animals fed organically was compared to pooled RNA from animals fed conventionally. Each sample was labelled twice, once with Cy3 and once with Cy5 (dye-swab). A single spotted chicken 20K oligo-array (ARK Genomics, Roslin, UK) was used. Hybridised microarrays were scanned using the Scanarray scanner and software (Perkin Elmer). Spot detection was done using GenePix Pro (Molecular Devices, Sunnyvale, CA, USA). A customised in house developed R-based normalisation procedure was performed to fit the data.[15] Subsequently, data were analysed using SAM.[16] Microarray data are available in the ArrayExpress database (www.ebi.ac.uk/arrayexpress) under accession number E-MEXP-1798.

Quantitative PCR analysis

cDNA was synthesised using Superscript II transcriptase kit (Invitrogen) according to manufacturer's instructions. Then, a quantity of 200 ng of RNA from individual chickens was diluted tenfold and 0,5 ug random hexamers were added. Reaction mixture was incubated at 70°C for 10 minutes. 4 μl transcription buffer, 2 μl 0,1 M DTT, 1 μl transcriptase, 1 μl dNTPS (2 mM each), 1 μl RNAsin (Promega, Madison, WI, USA) and 8 μl water were added. Reaction mixture was incubated at 37°C for 50 minutes, followed by 70°C incubation for 10 minutes. Primers were designed using Primer Express 3.0 software for Real-Time PCR (Applied Biosystems) based on the gene sequence that is represented by the oligonucleotide found to be regulated on the microarray. Primer sequences are listed in Table 2.

Table 2 Primer sequences

Gene Name	Accession No.	Primer sequence
Hemoglobin α chain	AY016020	Forward: TGCCAACACAGAGGTGCAA Reverse: GGGTCTCGGCGCCATAC
Acetoacetyl-CoA synthase	NM_001006184	Forward: AGCTGCTGGCACTCCTGAA Reverse: TCCTCCACCTTCGGAATCC
Isopenthyl-diphosphate delta isomerase 2	XM_418561	Forward: TGTGCAGAAGGATGTAACGCTTA Reverse: CGAGGCTTTGTCTAGAAGTTGCT
28S	DQ018756	Forward: CAAGTCCTTCTGATCGAG Reverse: TCAACTTTCCCTTACGGTAC

cDNA (2 μl) or colony material was used in a PCR reaction mix containing 5 μl buffer, 1 μl Expand High Fidelity Taq polymerase (Roche, Basel, Switzerland), 1 μl dNTPs (10 mM each), 1 μl forward primer (10 μM), 1 μl reverse primer (10 μM), 1 μl $MgCl_2$ (2 mM), 38 μl water. PCR program was as follows: 96°C for 5 minutes, 40 times (94°C for 1 minute, 58°C for 1 minute, 72°C for 30 seconds), 72°C for 7 minutes. PCR products were analysed on agarose gels. In case of colony PCR, reaction started with 96°C for 10 minutes to lyse the bacteria. PCR products were purified from agarose gel using QIAEX II gel extraction kit (Qiagen, Hilden, Germany) according to manufacturer's protocol. Purified PCR products were cloned into TOPO4 using TOPO TA Cloning Kit for Sequencing (Invitrogen). Cloned fragments were transformed to *E. coli* TOP10 cells (Invitrogen) according to manufacturer's protocol. *E. coli* containing TOPO4 – insert was grown in LB medium containing 100 μg/ml ampicillin and 50 μg/ml kanamycin overnight. Isolation of plasmid DNA was performed using the QIAprep Spin miniprep kit (QIAgen) according to manufacturer's instruction.

cDNA was diluted 10 times for qPCR analysis. Each reaction contained 12,5 pmol forward primer, 12,5 pmol reverse primer and POWR SYBR Green PCR Master Mix (Applied Biosystems) according to manufacturer's instructions. qPCR was performed using an ABI7500 (Applied Biosystems). The amount of *28S* was measured to control for variation in RNA-yield and RT-reaction conditions. In each run a standard curve was incorporated consisting of a vector (TOPO4) containing the cloned gene fragment. In this way both the gene expression and the external control gene expression could be related to a standard curve. The efficiency of the PCR reaction was 90-100% for all reactions (slope standard line between -3.3 and -3.6). The standard line consisted of 10-fold dilutions of the control vector. For each reaction negative water controls were included. Analysis was performed using the ABI7500 Software (Applied Biosystems). Statistical analysis on data was performed using independent students T-tests.

Results

Gene expression in chicken intestine after two different diets

Feed analysis has shown that the energetic value of both feeds was similar. Consistent differences existed in protein content, which was higher in conventional feed, whereas crude fat and ash contents were higher in respectively organic grower and starter feed, respectively (Table 3).

Table 3 Feed analysis

Nutrient	unit	Second generation			
		Starter		Grower	
		Conventional	Organic	Conventional	Organic
Energy	Kj/kg	14882	14729	15102	15231
Protein	g/kg	164	151	199*	176
Crude Fat, by acid hydrolysis	g/kg	42	42	53	62*
Carbohydrates, total	g/kg	620	624	574	585
Raw Fibre	g/kg	34	36	39	39
Moisture	g/kg	123	119	120	122
Ash Content	g/kg	51	64**	54	55

* ≥ 10% higher than the conventional or organic feed;
** ≥ 20% higher than the conventional or organic feed;

After 13 weeks conventional fed animals cumulatively consumed about 80 grams more feed compared to organic fed animals (3686 grams vs. 3607 grams). This difference in feed intake was statistically significant at age 12 and 13 weeks. There were differences in body weight of the animals. At hatching all animals showed similar weights between 32 and 35 g.At 13 weeks, in general, L-line animals gained most weight. Conventionally fed L-line animals reached a weight of 1209 $\pm$ 12 g and organically fed L-line animals reached 1209 (SD 21) g. H-line animals on the contrary were the lightest at the end of the experiment, where conventionally vs. organically fed H-line animals reached 1050 (SD 15) and 1048 (SD 18)g. Only among C-line animals did significant differences exist in body weight between the diets: conventionally fed C-line animals reached 1241 (SD 27)g, whereas organically fed C-line animals reached an end-weight of 1098 (SD 23)g. Extensive description of the analysis of chicken feed and physiological and immunological parameters of the chickens is described elsewhere (Huber *et al.*, Br J Nutr (2010),103:663-676).

To analyse the effect of diet on gene expression, all three chicken lines (H, C, and L) were analysed as one group, to minimise genetic background influence, with diet as only variable. After data analysis 49 genes were found to be significantly at least 3-fold regulated due to the different diets. Of those 49 genes, 28 genes were expressed higher in chickens fed conventionally grown ingredients, whereas 21 genes were expressed higher in chickens fed organically grown ingredients. The false discovery rate of those genes was 6.6%. The top 15 genes of up- and down-regulated genes, containing the genes with the strongest fold induction, is listed in Table 4.

Table 4 Genes that are regulated at least 3-fold in the jejunum of chickens fed conventionally grown feed ingredients compared with chickens fed organically grown feed ingredients independent of genetic background. The top 30 regulated genes out of 49 are shown.

Homology	Fold induction	q-value
Hemoglobin-α-chain	4.8	6.6
CCLi10	4.6	6.6
No homology	4.6	6.6
Chemokine ah221	4.2	6.6
Genome Hit Contig 1336.1	4.2	6.6
NDR-2 (weakly similar)	3.9	6.6
Early response to neural induction	3.9	6.6
Nuclear receptor (NroB2)	3.8	6.6
Insulin-induced gene 1 (Insig-1)	3.7	6.6
Immunoglobulin Heavy Chain	3.6	6.6
F-Box/LRR repeat protein 3A	3.6	6.6
Cytochrome P450	3.6	6.6
Hepatocyte growth factor like protein (HGFL)	3.5	6.6
Thrombospondin receptor (CD36)	3.5	6.6
No homology	3.4	6.6
Soluble carrier family 1	-3.2	6.6
α2-macroglobulin precursor α2	-3.3	6.6
Genome Hit Contig 190.26	-3.3	6.6
No homology	-3.3	6.6
No homology	-3.5	6.6
No homology	-3.6	6.6
No homology	-3.9	6.6
Hydroxysteroid (17 beta) dehydroxygenase	-4.8	6.6
Hypothetical Protein	-4.9	6.6
B – G protein precursor/MHC 3-G antigen	-5.1	6.6
C4 methyl sterol oxidase	-5.2	6.6
Isopenthyl-diphosphate-deltaisomerase 2	-5.4	6.6
Acetoacetyl-CoA-synthase	-5.6	6.6
Squalene mono-oxygenase	-9.3	6.6
Genome Hit Contig 41.179	-10.9	6.6

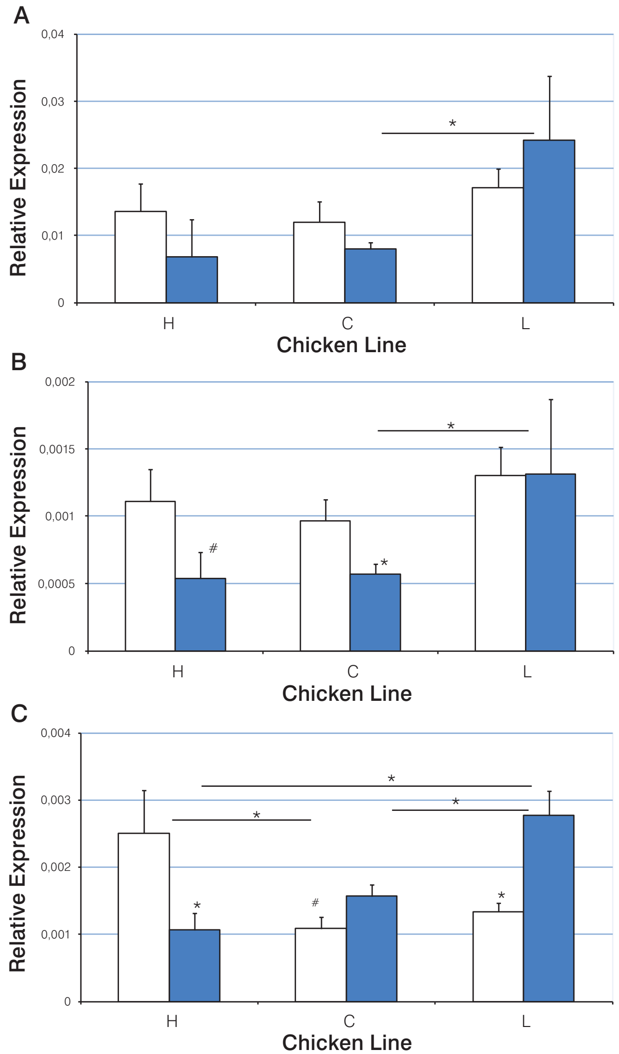

Figure 1

Effect of genetic background on diet-induced gene expression studied by quantitative PCR on three diet-regulated genes: acetoacetyl CoA synthase (**A**), isopenthyl-diphosphate delta isomerase 2 (**B**) and haemoglobin α chain (**C**). Three chicken lines were included H-line chickens with a high specific antibody response to sheep erythrocytes; L-line chickens with a low specific response to sheep erythrocytes; C-line control animals of randomly bred chickens. Clear bars: chickens fed organically, black bars: chickens fed conventionally grown feed ingedrients. Values are means of four to six chickens, with standard errors represented by vertical bars. * p<0.05; # p<0.1.

Quantitative PCR analysis of regulated genes in individual chickens of different chicken lines

To study the effect of genetic background on gene expression, expression of three diet induced genes was studied in individual animals of different genetic background using qPCR. One gene that was higher expressed in chickens fed on conventionally grown feed ingredients was selected (haemoglobin α chain) and two genes that were higher expressed in chickens fed on organically grown feed ingredients were selected, acetoacetyl CoA synthase and isopenthyl-diphosphate delta isomerase 2. Data were analysed using two parameters: diet (organically fed vs. conventionally fed), and genetic background (H-, C-, and L-line). Figure 1 shows that differences in gene expression between chicken lines as well as between diets were found. The unselected C-line animals showed gene expression patterns similar to the results of the microarray. However, animals from the selection lines (H- and L-line) showed different gene expression patterns. The H-line animals showed a higher expression of acetoacetyl CoA synthase and isopenthyl-diphosphate delta isomerase 2 in organically fed animals, which is comparable to the microarray results. L-line animals on the other hand showed a higher expression of acetoacetyl CoA synthase in conventionally fed animals, whereas isopenthyl-diphosphate delta isomerase 2 expression was not regulated at all. Haemoglobin α chain expression was found to be higher in conventionally fed animals of the C-line and L-line, but in H-line animals haemoglobin α chain expression was higher in organically fed animals.

Discussion

In the present study we demonstrated transcriptional differences between jejunum of chickens fed on two diets, identically composed out of organically grown or conventionally grown feed ingredients. Forty-nine genes were differentially expressed at least three-fold between chickens on the different diets. Of those 49 genes seven genes were directly or indirectly involved in cholesterol biosynthesis. qPCR analysis revealed differences in the way genes are regulated between the different chicken lines. Thus feed regulates gene expression independently of genetic background of the chickens, but the genetic background influences to what extent feed regulates gene expression. Our microarray data yielded 49 regulated genes between chickens fed on the different diets with a false discovery rate of 6.6%. Compared to other microarray experiments this is a low number of regulated genes with a low statistical power. The clustering of regulated genes in the pathway of cholesterol biosynthesis strengthens the power of our microarrays. However, considering the fact that both chicken groups are healthy chickens in good conditions, fed on the same food ingredients, this small difference in gene expression was expected. It can be debated how comparable both feeds actually were. Although, it was attempted to compose diets of the same ingredients, with the same energetic value, it is clear differences existed between the feeds as shown in Table 2. In this study it was decided to collect ingredients from neighbouring conventional and organically farms. Since both agricultural systems have their own varieties suitable for their specific system, it had to be accepted that different varieties were used.[17-19] Besides, it is known in both conventional and organic farming large differences exist between farms. Still, this approach was chosen because the full system of either conventional or organic farming is represented, both systems using their own specialised variables. Differences in gene expression can thus be attributed to differences between farming systems.

Seven genes that were differentially regulated between the two feed groups, independent of genetic background, are directly or indirectly involved in cholesterol biosynthesis. Figure 2 shows a schematic representation of cholesterol biosynthesis. Five genes directly involved in this pathway were found to be lower expressed in the chickens fed on conventional ingredients (underlined in Figure 2). Two other regulated genes were indirectly involved in cholesterol synthesis. Insulin induced gene 1 (*insig-1*) and P450 were both higher expressed in chickens fed conventionally. Insig-1 is a key regulator in cholesterol synthesis that forms a complex with sterol regulatory element binding protein (SREBP) and SREBP-cleavage activating protein (SCAP) in presence of cholesterol. When this complex of SREBP-SCAP-Insig-1 exists, SREBP is repressed in its transcription activation of cholesterol synthesis (reviewed in[20]). Overexpression of *insig-1* will thus repress cholesterol synthesis. P450 is directly involved in steroid synthesis, but also acts as a negative feedback mechanism to shut down cholesterol biosynthesis (reviewed in[20]). Upregulation of *p450* will therefore result in downregulated cholesterol synthesis. In conclusion, regulation of these seven genes involved in cholesterol biosynthesis lead to less cholesterol synthesis in the jejunum of chickens on the conventionally grown feed.
Cholesterol synthesis is tightly regulated by several factors. The strongest regulator of cholesterol biosynthesis is circulating blood cholesterol itself. However, no differences were found in circulating cholesterol levels between animals from the two diet groups (Huber *et al.*, manuscript submitted to BJN). Food analysis revealed that the organically grown diet contained more crude fat compared to the conventional grower diet (Table 2). This difference in constitution between the two diets could lead to differences in cholesterol biosynthesis. Conventional feed on the other hand contained slightly more calculated phytosterols compared to organically feed (data not shown). A correlation exists between phytosterols and cholesterol metabolism that among others acts through the SREBP pathway.[21] Phytosterols lead to lower cholesterol levels, so the differential regulation of cholesterol biosynthesis pathway could also be attributed to this difference in feed composition. All these factors together might explain the differences observed in the cholesterol synthesis.

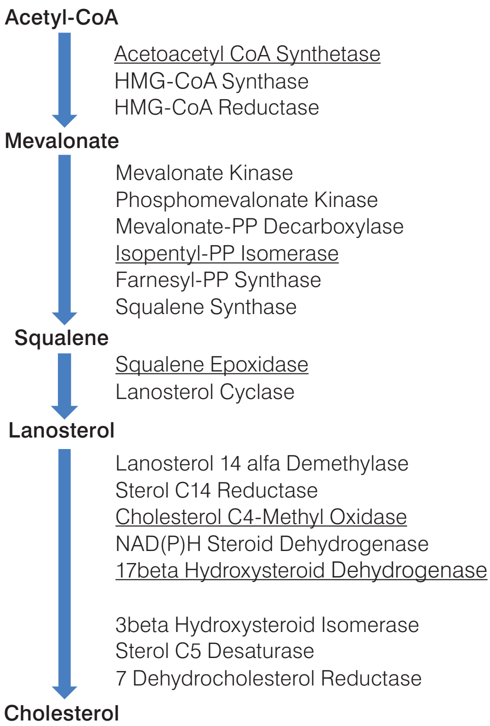

Figure 2

Schematic representation of cholesterol biosynthesis freely adapted from Espenshade & Hughes[20]. Underlined genes were found to be lower expressed in the chickens fed on a diet of conventional ingredients. HMG, hydroxyl-3-methylglutaryl; PP, diphosphate.

Although the genes in the cholesterol pathway are higher expressed in the organic fed chickens, these chickens had lower body weight. We do not know however if the end product of the cholesterol pathway is cholesterol or for example steroid hormones. Therefore it is hard to relate body weight to the observed differential gene expression. Taken together, microarray results showed that cholesterol synthesis is differentially regulated between the two feed groups. The exact trigger for this regulation is unknown.

Three other diet regulated genes seem to be involved in immunological functions: chemokine ah221, B-G protein precursor and immunoglobulin heavy chain. Chemokine ah221 (homologous to human MIP-A1) is higher expressed in conventionally fed chickens. This chemokine is involved in innate immunity and promotes chemotaxis of T lymphocytes. B-G protein precursor is higher expressed in organically fed chickens.

B-G protein precursor is part of the major histocompatibility complex of the chicken, and is strongly correlated with disease resistance in chicken[22]. Immunoglobulin heavy chain is higher expressed in conventionally fed chickens. Although one would expect the immunoglobulin light chain to be higher expressed as well, only little is known of B-cell development in the avian gut. Therefore, it is hard to interpret overexpression of just the immunoglobulin heavy chain. The regulated expression of genes involved in immunity at least indicates there are immunological differences between the different diet groups of chick. This observation is confirmed by differences in specific and innate cellular and humoral immune responses in birds fed organically and conventionally grown food described elsewhere (Huber *et al.*). The synergistic relationship between diet and the immune system was already described in the 1960's as reviewed by Scrimshaw.[21] Recently, the field of epigenetics seem to explain at least part of those dietary effects. *In utero* exposure to a methyl rich diet can enhance the severity of allergy airway disease in the offspring through changed methylation of specific genes[11]. Since in our study design, both maternal and offspring animals were fed the same diet, epigenetic changes in the genome of the offspring due to differences in diet of the mother hens cannot be excluded. The resulting changes in gene expression may therefore already have been induced in maternal animals and subsequently transferred to the offspring. To test this hypothesis, epigenetic studies on both generations are required. It is hard to predict if the differential expression of immunological genes might have an effect on disease resistance or health in either one of the groups. Further research including a challenge experiment with a pathogen, as well as connected clinical observations on the animals, are necessary to draw conclusions regarding the effects of these regulated genes.

To investigate the effect of genetic background on the genes that were regulated by diet, three independent chicken lines were separately investigated by qPCR. The chickens studied originated from lines that as a consequence of genetic selection differ in almost every aspect of innate as well as specific immune responsiveness.[23-26] Such lines enable estimation of advantageous or negative effects of diet and health risks with respect to genetic background. Gene expression of three differentially expressed genes, found by micro array analyses, that were diet dependent was analysed. qPCR analysis on individual chickens revealed that the three chicken lines used in this study did not behave uniformly. Two out of three genes were regulated in all three lines. However, those genes were higher expressed in conventional fed animals of the one line, whereas they were higher expressed in organically fed animals of the other line. Expression of the third gene was regulated in two out of three lines, but not regulated in the third line. These data show that besides dietary effects, genetic background of chickens can also affect the transcriptional response to diet (components).

In this study we describe that there are transcriptional differences in the jejunum of chickens that were fed different diets. Forty-nine genes were differentially regulated between chickens fed a diet from organically grown feed ingredients, compared to a diet from conventionally grown ingredients. Although differences in mRNA expression levels are not necessarily correlated to protein expression levels or physiological effects, it is the first time that significant differences in gene expression were shown between animals on identically composed diets from conventional and organic origin. Based on our data it is impossible to predict the implication of those differences, let alone decide which diet is more healthy or beneficial for the chickens.

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A. R. initiated the project at the Central Veterinary Institute and revised the manuscript. A. G. conducted and analysed the microarray experiments and drafted the manuscript. W. S. assisted with the animal experiment and conducted qPCR analyses. H. P. genetically selected the chicken lines that were used in this study. L. V. described the food analysis. M. H. is project leader of the project “Organic, More Healthy?”, and assigned part of this project to the Central Veterinary Institute.
The authors state that there are no conflicts of interest.

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Chapter 11

General discussion

Introduction

The preceding chapters of this thesis deal with two seemingly different topics; namely, the definition of health, on the one hand, and the research on the health effects of specific foods, on the other. However, there is an underlying theme that connects both topics. This theme is about the aim to elaborate an integral concept of health, in a modern and scientific way, connected to the integrity of life and offering a context for the interpretation of health research results, from a broad perspective. This thesis gives a concise description of the knowledge development on health as well as nutrition – from a historical qualitative approach that considered the 'wholeness' of health and food, towards the analytical and quantitative approach that forms the basis of modern science. The result of this analytical approach is a huge amount of factual knowledge, on an increasingly more detailed level, but with the risk of losing sight of the organism as a whole, be it a human being or a food product. However, the modern collaboration between analytical methods and ICT in so-called omics methodologies, combined with physiological knowledge, brings forth new opportunities for perceiving, once again, the wholeness of organisms and systems, but on a much higher level of knowledge than in ancient times.[1] This thesis is focused on the development and evaluation of a broad and integrated perspective on health and nutrition, in both a conceptual and an analytical way.
This pursuit of knowledge on an integral and systems level can presently be observed in different scientific disciplines. For example, Rockström *et al.* discuss the resilience of the earth[2], Holland *et al.* describe complex adaptive systems[3] and Scheffer *et al.* present factors that influence or disturb the stability of systems.[4] These authors represent a movement in science and its applications that shifts from a focused one-issue approach towards a multifactorial systems approach, and that deals with concepts such as integration, resilience and self-regulation.
Schematically, this transition towards an integrated approach is visualised in Figure 1.

This thesis aims to contribute to this transition in science and its applications.

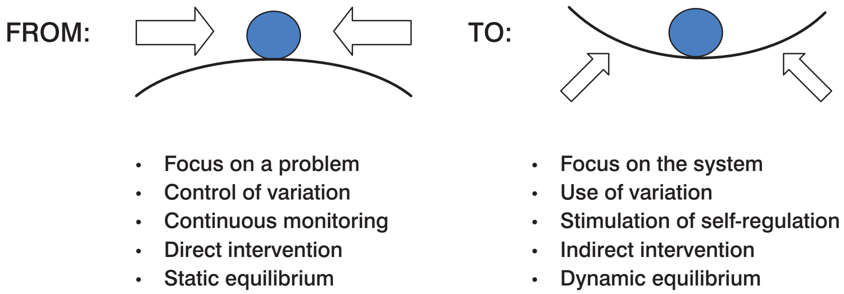

Figure 1 Visualisation of the transition in science and its applications, from a focused and specialised approach towards an integral model, as is needed for the creation of resilient and self-regulating systems[5]

Main findings

The first research question:

1. *Health: How has this basic value for mankind been defined in history, by physicians as well as philosophers, until recent times? What were the intentions of the WHO, when defining health in 1948? Could the WHO intentions be maintained, albeit in a new, more dynamic conceptualisation of health? How is this newly proposed concept of health evaluated by the various stakeholders in healthcare, and how could it be further operationalised for the future?*

In Chapter 2 we described that in ancient times, *health* (etymologically) was perceived as 'wholeness' and specified as a *balance* of qualities, with *illness* being considered a disturbance of this balance. Hippocrates, for example, considered health to be a balance of four 'humours', which together constituted the human being, whereas Aristotle considered that addressing health by maintaining the 'mean' was a virtue, as was personal development towards '*eudaimonia*', nowadays translated as flourishing. During the Renaissance and Enlightenment, the physical bases of diseases were explored, ultimately resulting in the concept of health being the equivalent of '*the absence of disease*'. The related analytical approach appeared very fruitful and resulted in today's modern science, with a vast biomedical body of knowledge and ongoing specialisation in the medical profession towards ever more detailed levels. With the establishment of the WHO in 1948, a very broad and idealistic definition of

health was formulated, addressing the complete well-being of the human being on a physical, mental and social level. The WHO definition was intended as a goal to work towards, for the happiness and well-being of the world population. Yet, from the start, there has been criticism of this definition, mostly concerning the word 'complete'. Since 1948, many different formulations of the definition of health have been proposed, about which Tarlov in 1996 concluded that three components were mentioned markedly consistently: 1) The capacity to perform (relative to potential); 2) The achievement of individual fulfilment; the pursuit of values, tasks, needs, aspirations and potential; 3) In a social environment, good health provides the potential to 'negotiate' demands of the social environment.
Reflecting from this perspective on our concept of health as 'the ability to adapt and to self-manage, in the face of social, mental and physical challenges of life' and its elaboration into the broad concept of 'positive health', consisting of six dimensions (Chapter 3 and 4), we conclude that these cover the conceptual components identified by Tarlov (Chapter 2): Our concept is active, addresses potential, is individualised, includes a person's fulfilment and values in the spiritual/existential dimension, and contains diverse social skills and aspects in the social/societal dimension.

Moreover, we think that the intentions of the WHO – to address ultimate human well-being – are still fully represented in the above formulation.
When testing the support for this new concept of health, among approximately 2000 different stakeholders in healthcare in the Netherlands, a fair number of differences were found in their interpretation of the content of health (Chapter 4). A 'broad' interpretation, according to which all six dimensions were valued as almost equally important to health, was shared by patients with a broad diversity of diseases. To them, health was about 'life as a whole'. This opinion was not expressed by physicians, among others, who chose a narrower and mainly biomedical interpretation of health. This apparent conceptual gap between the two groups that, principally, dependent on each other in cases of disease, was a finding that required special attention. Indeed, if the policy principle of 'patient-centred care' is to be taken seriously, this outcome has consequences for the physician and his/her approach to the patient. The relationship between physician and patient has not lost its topicality, as the Lancet showed recently when citing the view of French physician Canguilhem. In 1943, he stated 'Health is not defined by the doctor, but by the patient, according to his or her functional needs. The role of the doctor is to help each patient adapt to their unique prevailing conditions.'[6] In 1983, Dutch physician Knottnerus similarly argued that 'it should not be the physician but the patient, with his individual experience of well-being and healthiness, who decides what health is. It is the role of the physician to identify and, together with the patient, combat the factors that hinder the patient's specific feelings of well-being and health'.[7] (Chapter 2). Both authors proposed a

clear patient-oriented and integrated approach, which can still be a guidance in medical training and in practicing 'shared decision-making' today.
Another contrast was found between policymakers and researchers, with their 'narrow' focus, and patients. This might be understood from the way governance is generally organised, with strict separations between domains such as healthcare and social affairs. However, another contrast that also drew our attention was connected to the level of education. Having a university degree appeared to be connected to a more narrow and biomedical perception, even among the group of 'patients', compared to all other levels of education. One explanation could be that policymakers and researchers will often have a university education, which may also have contributed to the described contrast. Another explanation could be that the content of a university education does not easily relate to the way the general public and patients experience daily life.
To avoid confusion between different interpretations of 'health' – being either the still common view of health as 'the absence of disease' or the broad interpretation of health being about 'life as a whole', the concept of 'positive health' is being proposed for the broad interpretation (Chapter 4). We consider 'positive health' to be the bottom-up induced, first step in the operationalisation of the new concept of health.
In 1946, during the preparations for the WHO definition, the term 'positive health' was already mentioned, but ultimately not chosen. The present concept of 'positive health' is defined by the six dimensions and visualised in a spider web diagram with six axes by which the dimensions all appear equally important. This concept and its visualisation makes it possible to perceive health as wholeness, as patients prefer, but without denying the importance of disease and its treatment. In fact, the domain of health potential, and the domain of pathology and treatment can exist as a 'two continua model'.[8] In the web diagram, disease will always reveal itself; for example, in the dimensions of *bodily functions* or *the mental functions & perception, albeit within the context of abilities.*

The second research question:

2. *Nutrition: It is a precondition for human life and functioning and, from various perspectives, poses challenges for mankind. How has nutrition been perceived and studied in history, until recent times? What does the newly proposed dynamic concept of health mean for the evaluation of health effects of foods in general and, more specifically, of organically grown foods that are produced according to a systems approach?*

We described in Chapter 5 that the perception of nutrition, from ancient times up to the present, showed a development congruent with that of medicine. A paradigm shift occurred from the 16th century onwards, towards an analytical way of thinking, which also affected food production and resulted in modern industrial agriculture and food processing, in large parts of the world. Yet, a little over a century ago, also a critical countermovement emerged among consumers and shortly after that they were joined by farmers, who emphasised that they considered both human beings and food products as living entities and more than merely 'the sum of the parts'. An agricultural approach of ecological systems on farms developed, with self-regulating properties, aiming at balanced and resilient soil life, plants and animals. The hypothesis was that food products from such a source would strengthen the health of consumers. Until recently the two visions of agricultural production approaches mainly collided in emotional debate, but modern analytical techniques may help to overcome the emotions and provide insight in the relations between agricultural approaches and their impact on crops and animals.

In nutrition, modern omics research techniques present new possibilities to investigate relationships between nutrition and health, as we described in Chapter 9 and 10. They support the already existing physiological knowledge that different nutrients interact within the organism of the consumer. These techniques show that food products contain thousands of compounds, which shift in 'clouds' related to the production system, whereas within the physiology of the consuming organism they also show a richness in patterns, which become influenced by the consumption of food. The large amounts of available data challenge the researchers' interpretation. Here, phenotypic observations and knowledge about the physiology of humans, animals and plants, combined in systems biology, will prove an indispensable basis for such interpretation.[9,10,11]

This modern development in the field of nutrition might enable a paradigm shift, similar to what might occur in the field of medicine, towards once again perceiving 'wholes' (whole products, organisms), but on a much higher level of knowledge than in ancient times.

For this thesis, we attempted to connect research on the health effects of food to the new dynamic concept of health. If 'ability to adapt' is connected to 'health', a logical step is to design research that includes challenges of different kinds, in order to study the way organisms deal with these challenges, and to question if homeostasis is being regained easily. Such an approach could also produce insights into whether certain production measures have an impact on the resilience or 'phenotypic flexibility' of an organism, as expressed in the coherence in recovery of the various physiological processes.[12]

The previous chapters (8 to 10) describe the application of this approach in the study of possible health effects of organically produced food. Organic food production can be considered as the currently most commonly used systems approach in animal and crop farming, with the aim of maintaining the natural resistance of plants and animals, and excluding both pesticides and the preventive use of antibiotics. The question about possible beneficial effects related to the consumption of products from this production system is a topic of high societal interest, but also one of scientific controversy. The Chapters 6 and 7 describe the state of the art in research on this topic, with different types of traditional nutritional research – analyses and consumption studies – as well as the difficulties connected to the design of such studies. It was concluded that, based on the available research, there are indications of a beneficial effect on health, but the differences in quality between organic products is large, and the influence of the annual climatic conditions is very high.

The animal study described in this thesis (Chapter 9 and 10) – an immunological chicken model used as a model for humans – with either organically or conventionally produced feed in a fully controlled and blinded situation, was the most comprehensive study performed to date, worldwide. An immunological challenge was applied in young animals of the second generation, which all appeared perfectly healthy. In all animals, this resulted in suppressed growth and a strong immunological and metabolic reaction, ultimately followed by catch-up growth as a sign of recovery. The two groups showed striking and statistically significantly different reactive patterns, besides already differences in immune titres after vaccinations before the challenge. The group fed on organic feed reacted more strongly, both immunologically and metabolically, to the immunological challenge, and showed an earlier onset of catch-up growth. In this study's publication of 2010, this coherent reactive pattern was described as more 'resilient'. This effect could also be described as showing a stronger ´phenotypic flexibility´[12] and it could be argued, based on Chapters 3 and 8, that this pattern is 'healthier'.

After the challenge, also genomics was performed on the gut of the animals. Although 49 genes were regulated significantly differently between the feed groups, at the time we were unable to interpret the observed phenomena. Nevertheless, it was an indication that also on a gene level, differential effects of the feed from different production systems on animals could be detected, which is an epigenetic phenomenon. Overall, the 2010 study also confirmed the informative value of a metabolic or immunologic challenge as part of a research design.

Furthermore, this also illustrated the well-known fact that, in nutrition research, studying the effects of consumption on living organisms is far more informative than a mere chemical analysis of ingredients.

Strengths and limitations

Regarding the strengths and limitations of this thesis, it can be concluded that the broadness of the discussed topic is evidently both a strength and a limitation. A strength is that the content sketches overviews and addresses generic issues that are presently most current, and that an attempt is made to develop viewpoints that may support further development of the integral knowledge on the various issues. Yet, the broadness and complexity is also a weakness and a pitfall, as the contribution to the themes was only limited, leaving many details undiscussed. The following section sketches a framework that differentiates this broadness, and presents the next steps for further operationalisation of the theme on the health topic.

A strength is the formulation of a new, dynamic concept of health: 'Health as the ability to adapt and to self-manage, in the face of social, physical, and emotional challenges'. A further strength is that the empirical survey included more than 2000 stakeholders in Dutch healthcare, who evaluated the concept and valuated it very positively. In the search for indicators of health, these were induced bottom-up instead of being designed behind a desk; this could be seen as an additional strength. The content of indicators in the specific domains, of what we finally overall called 'positive health', was formulated in a consensus process involving experienced researchers from the NIVEL Institute.

The novel results from the evaluation in the quantitative survey can also be considered as a strength of this thesis. These results showed patients' broad perspective of health, as well as the physicians' different interpretation of health, and thus indicated the risk of misunderstandings in the interaction between the two, in medical practice; this is, therefore, a point of attention. The even bigger difference in interpretation between patients on the one hand and policymakers and researchers on the other – the latter group being the professionals that design and evaluate the medical systems for the former – is also an important sign.

The deliberate choice was made to approach the question about indicators of health in an open way – similar to the earlier process of formulating the general concept – and to subsequently ask the interviewees whether their described indicators matched the general concept; this was confirmed by three-quarters of them.

A weakness, however, is the fact that we did not test if the described 6 dimensions and 32 aspects of health indeed matched the 'ability to adapt' and the 'ability to self-manage'. This still needs to be done.

Another weakness is that the evaluation was only conducted in the Netherlands and its generalisability to other, especially non-western populations, cannot be guaranteed without further research.

Furthermore, the WHO have indicated that they would not consider to replace its current definition by this new formulation, as long it could not be objectively measured, which to date it cannot. A strength, from this perspective, is that we compared our findings to the International Classification of Functioning, Disability and Health (ICF)[13], WHO's present terminology for health, functioning, and health-related domains with multi-dimensional concepts. Together with an ICF specialist, we linked the aspects of our concept of 'positive health' to the most appropriate ICF categories using the linking rules that WHO advises. Using the ICF classifications, we were unable to categorise and recode many of our positive health indicators into ICF. One important reason is that the ICF lacks a classification for *personal factors*, and many of our aspects seemed to best suit this category; 18 of the 32 indicators were coded as such. This can be considered a challenge for the future, for ICF and WHO, as well as for the development of our concept of positive health with respect to measurability. When addressing nutritional research, the first steps towards operationalisation of the new concept for effect studies by using challenges to study resilience, can be considered a strength. The overview of possible challenges, as well as the example of the intervention study with the immunological chicken model on two feed types, clearly illustrated how such research can be designed and implemented. Nevertheless, it could be considered a weakness that the described intervention study was performed using chickens, which may not to be the first model of choice for humans. Chickens do not have the differentiated organised lymph nodes of mammals, but apart from this, the avian immune system is generally considered quite comparable to the mammalian one, and it has provided the basis for a large amount of the current knowledge on the immune system. We chose this model to start with, for its reliable immunological aspect and because it was available at a nearby location within certified surroundings. The size and full scientific setting of our intervention study could be seen as a further strength. The study showed significantly different effects for foods from various production systems on consuming organisms, on an integral phenotypic level (as demonstrated by the occurrence of catch-up growth), as well as on levels of immunology, the metabolome and the genome. Another major strength is the fact that neither the conventional nor the organically grown food contained traces of pesticides, which are usually hypothesised to be a cause of possible differences in the health effects of foods from different production systems. Another strength was that the ingredients of the feeds, from the two agricultural systems, were analysed extensively; macro- and micronutrients, i.e. vitamins, minerals, trace elements, heavy metals, microbes and residues of pesticides. Had the comparative study on nutritional value of these products from two agricultural systems been restricted to just these analyses, the conclusion would have been that no differences occurred that would suggest health benefits, as the analysed differences between the ingredients were not very large, except for a 10% higher protein content in the conventional feed. However, metabolomics allowed us afterwards to distinguish ingredients[14], and also the effects of the consumption of these feeds by the animals showed clear differences; the chickens fed on organic feed showing a lower body weight, an enhanced immune response on vaccinations, a stronger reaction to the immune challenge, as well as a stronger catch up growth after the challenge than the group on conventionally produced feed. These results support a systems approach in nutritional research, in analysing ingredients, as well as in studying the effects from consumption.

Implications of the study

HEALTH

The new health concept and Dutch society

Since this research was performed, the Dutch Government initiated three large transitions in the societal organisation of social support and healthcare. One of the underlying reasons for these transitions is that the costs of the present healthcare system are expected to be no longer affordable in the future, when a 'silver tsunami' of elderly will flood society. These transitions are characterised by a shift towards more personal responsibility of citizens for their own well-being, more participation in society and more dependence on their social networks. The societal organisation of support and care will become decentralised and more locally organised. And a reorganisation of financial structures will be realised, making prevention more profitable.[15]

In line with this development, the new concept of health as 'the ability to adapt and to self-manage, in the face of social, physical, and emotional challenges' was recognised as potentially congruent with the aims of the Dutch Government. The newly installed Committee Innovation Healthcare Professions & Education[16] of the National Health Care Institute, while unaware of the results elaborated in our follow-up study (Chapter 4), initiated a qualitative investigation by the Verweij-Jonker Institute, in which, in 2013, the new health concept and its consequences were discussed in 28 focus groups among a broad range of stakeholders in healthcare. These stakeholders mostly included the same groups we had investigated, in an even more differentiated way, but lacked the insurers and a representative group of citizens.[17]

Their investigation turned out to be an external validation of our qualitative study, regarding the support among stakeholders for the new concept. The questions posed focused on evaluation of the concept, consequences for citizens, actions needed to support these citizens, and how to realise an implementation of the concept in the healthcare system. Similar to our study, the concept was evaluated positively; it was recognised as relevant that, for cure and care, the focus should be on functioning, resilience and self-management, and that health should not be regarded as a goal in itself, but rather as a means to a meaningful life. As in our study, there were reservations about all people being capable of this. A newly mentioned point of attention was that of the concept bringing the risk of a dichotomy in society, with people following a healthy lifestyle no longer willing to pay for those making unhealthy choices.

The other questions studied in that investigation are not discussed here.

Before we describe some thoughts about further operationalisation of our broad concept of health, first we explain the choice for 'positive health'.

The choice for 'positive health'

Why 'positive health'? A first and obvious objection would be that health in itself is a positive concept. However, as described in Chapter 2, health originally was connected to *wholeness*, *integrity* and *balance*. Over time, this content of health was replaced, in the context of a disease- and risk-focused medical culture, by that of the *absence of disease*. During our interviews (Chapter 4), respondents indicated that the new concept of health implies a paradigm shift, from a disease-oriented focus of healthcare to a health-oriented salutogenic focus, and that we should be aware of the risk of confusion if we would apply the word ´health´ in this context. In the interviews, the term ´positive health´ was suggested as the name for our new concept. We were acquainted with the movement of ´positive psychology', which aims to develop people's strengths, besides providing therapy for psychopathology. In positive psychology, Aristotle's *eudaimonia* is the central concept. Eudaimonia in modern translations is described as happiness and personal well-being and connected to the strive for meaning, for fulfilling one's potential, and to flourish. Such elements are also included in the broad interpretation of health among patients (Chapter 4). We recognised a congruence between elements from positive psychology and aspects in the mental, spiritual/existential, quality of life, and social/societal dimensions of our broad concept, which was one of the reasons for choosing the term 'positive health'. Another reason was

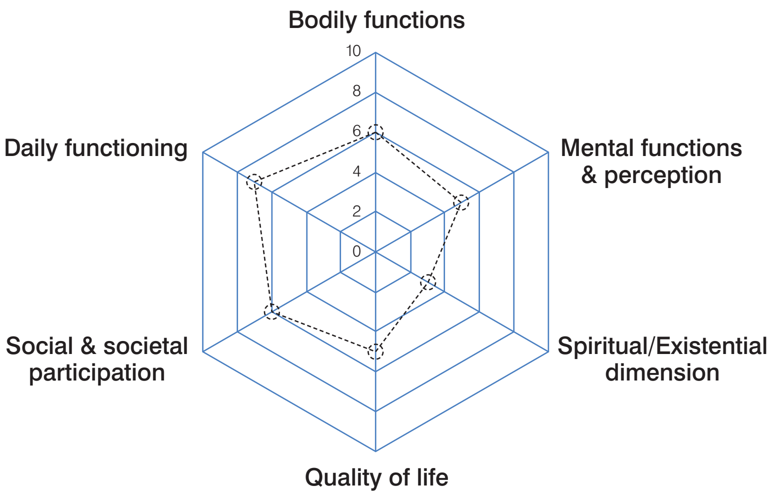

Figure 2 The 6 dimensions on a subjective scale, visualised for practical use, representing a fictional estimation of a person's state of 'positive health'

that 'positive health' was already mentioned during the preparations of the Constitution of the WHO in 1946.[18] We investigated how the term had already been used in the literature, which mostly appeared to be rather vague. We decided to choose 'positive health' in connection to the 6 dimensions and 32 aspects of health that we elaborated in our research, and the spider web diagram representation (Chapter 4, Figure 2).

Further development of 'positive health'

We elaborated a first step towards operationalisation of the new concept of health. However, the concept needs further elaboration. In that respect, a framework specifying topics to be developed may be helpful. Below, an overview is presented, followed by a more detailed reflection.

A framework of specific topics related to further operationalising of the concept of 'positive health' would imply the following:

1. Differentiate between *positive health* and *the domain of curative medicine,* for reasons of giving balanced attention to both, in a *two continua model* of health and disease, across the human lifespan.
2. In addition to the regular content of '*prevention*' – *the three Ps:* health *protection* against external risks, *prevention* of diseases and risky behaviour, and health *promotion* in the sense of promoting conventional healthy behaviour – also add the promotion of *positive health.*
3. Concerning *determinants* of health: differentiate between levels where *individuals* can create health-improving environments for themselves, and where this is an overarching *public responsibility*. Concerning *individual determinants* of health: differentiate between *external* and *internal resources* for health and provide *education* about these resources. For adults, but also in schools.
4. *Indicators/outcome measures* will be needed to assess levels of positive health and to evaluate the development. Differentiate between health on both an *individual* and *population level,* and between indicators/outcome measures that are *self-reported and subjective* and those that are *externally acquired and objectivised.*
5. Concerning the *training of health professionals*: for professionals, in addition to training in pathology and treatment, also include familiarity with a broad perspective on health, and knowledge about positive psychology, training in personal resilience and self-management, as well as skills for shared decision-making and motivational interviewing.
6. *Research* remains important, whereby clinical research for *evidence-based medicine* needs to interact with *practice-based evidence* from clinical practice, in order to connect to the reality of the practice.

Some reflections on the framework topics, related to operationalising the concept of 'positive health'

1. Differentiate between *positive health* and the domain of *curative medicine* for reasons of giving balanced attention to both, in a *two continua model* of health and disease, across the human lifespan.

Antonovsky (Chapter 2), initiator of the salutogenesis approach, described health and disease as two extremes on a continuum. This would imply that disease excludes health and thus health is the absence of disease. We prefer a *two continua model*, as described for mental health and psychopathology[8], which assumes health and illness are related but distinct dimensions, and can occur simultaneously. Both need attention and development, but in a more balanced way than in current practice. In 2013, 96% of the Dutch budget for healthcare went to cure and care and 4% to prevention. The domain of cure and care could well benefit from the application of elements derived from the other domain, as there is ample literature available about the influence of psychological strengths on the physically ill; for example, about the effect of resilience.[19]

2. In addition to the regular content of '*prevention*' – *the three Ps:* health *protection* against external risks, *prevention* of diseases and risky behaviour, and health *promotion* in the sense of promoting conventional healthy behaviour – also add the promotion of *positive health*.

Prevention is mainly the field of public health professionals and organisations. In the survey (Chapter 4), there was the least contrast in the participants' view on health between the group of patients and this group of public health professionals and organisations (as well as the group of the nurses). If an expansion towards promotion of positive health is to be realised, it would be quite logical if this stakeholder group would engage itself in developing this theme professionally, as a part of their efforts related to prevention. Here, it should be kept in mind that capabilities and motivations of individuals differ, and that different approaches should be developed for these different levels. In addition, this prevention does not need to be restricted to these professionals, but should become a skill of all cure and care providers.

3. Concerning *determinants* of health: differentiate between levels where *individuals* can create health-improving environments for themselves, and where this is an overarching *public responsibility*. Concerning *individual determinants* of health: differentiate between *external* and *internal* resources for health and provide *education* about these resources. For adults, but also in schools.

During our interviews, patients several times indicated that the indicators of health they mentioned were to them also determinants of health. However, this was not our question and this area of determinants and interventions to increase positive health

still needs to be developed in a sound and integrated way. In the Netherlands, the Centre for Healthy Living, which studies the evidence concerning health promoting interventions, could be a source of input of validated interventions.[20] Further, e-health tools are being developed, for example, by the Trimbos Institute[21], as well as self-management trainings by the CBO.[22] In the United States, Seligman developed resilience programmes for adults and school children, which may offer input for the development of interventions aimed at resilience development in the Netherlands.[23] However, not all input will come from institutions. Self-help networks are emerging and, especially when they are connected to a certain topic, citizens or patients can enter into fruitful exchanges of their experiences.[24,25]
The domain of employment or volunteer activities by adults also can be a source of health enhancement. If the living and working environment is designed in such a way that it invites healthy behaviour, more can be gained. It is therefore that within the ICF, the domain of 'environmental factors' has been included, partly focused on risks, partly on health-promoting aspects.[13]
With the described transition towards decentralisation, the design of an inviting environmental context – be it a healthy food environment, an exercise-friendly environment, or a safe social meeting environment – becomes even more urgent for local government. This field of expertise also needs further development.

4. *Indicators/outcome measures* will be needed to asses levels of positive health and to evaluate the development. Differentiate between health on *both an individual* and *population level* and between indicators/outcome measures that are *self-reported and subjective* and those that are *externally acquired and objectivised*.

At a glance, it seems evident that a person's estimation of his or her positive health differs from what a public health monitor would be interested in on a population level. However, positive health with its 32 aspects, to a large extent, contains subjective qualities that can best be self-reported. It is, after all, questionable if a measurement tool for use on population level needs to be very different from one that is used individually, or by a general practitioner. It could very well be that the presently developing knowledge concerning Patient Reported Outcome Measures, PROs and PROMs, will prove to be very applicable to developing a measurement instrument.[26,27] In addition, the ICF instrument may be used as a starting point in the assessment of the functioning of various aspects.[13]

5. Concerning the *training of health professionals*: for professionals, in addition to training in pathology and treatment, also include familiarity with a broad perspective on health, and knowledge about positive psychology, training in personal resilience and self-management, as well as skills for shared decision-making and motivational interviewing.

As described before, our results showed a large difference in the interpretations of health between the healthcare providers, especially the physicians, and the patients. In professional training this should be a topic of attention, and the professionals should become familiar with the broad interpretation of patients, as well as with the phenomenon of 'response shift'[28], which occurs when people experience a major life event (Chapter 4). Professionals should not develop only professional medical and communications skills. To be able to interact with a patient on an equal level and for real 'shared decision-making', ideally also personal development should receive some attention; for example, skills for personal resilience and self-management, knowledge on the basics of positive psychology, as well as motivational interviewing could be part of the educational programme.[29] Such a training may benefit patients as well as care providers. Recent studies showed that, among Dutch physicians, 20% had suffered a moderate to severe burn out, whereas among medical professors this was 25%.[30,31] A professional training that includes personal development may offer some protection to burn out, as it connects the care provider more strongly to his own motives as well as providing him with the skills to remain balanced.

6. Research remains important, whereby clinical research for *evidence-based medicine* needs to interact with *practice-based evidence* from clinical practice, in order to remain connected to the reality of the practice.

As citizens become more empowered, which is the aim of the Dutch Government, they will claim more freedom to make their own choices concerning treatments and interventions. Although more empowerment and self-direction can be considered a positive thing, it nevertheless will remain important to evaluate the effectiveness of interventions and to disseminate the conclusions. Here, the need for interaction between clinical research designs and clinical practice, with learning from informal evidence, will remain very important, in order to maintain physicians' and patients' trust in the value of evidence-based medicine.[32,33] If, as in positive health, the focus is not on disease but on enhancing health and resilience, one important research topic will be to establish markers for health. This thesis describes the value of research with applying challenges (Chapter 8), aimed at estimating the organism's ability to restore homeostasis or allostasis. This is only the first step. The search to establish markers for 'health' and for the range within which an organism is still capable of restoring a physiological balance, and where 'pathology' starts, is still in its infancy, but the already mentioned omics techniques could prove helpful in this process. In the literature, the concept of 'health space' has been introduced to describe an organism's physiological range of resilience. The perception is that, once a critical boundary of this health space is has been crossed, disease develops.[34] A connected question will be that of why a balanced system passes a 'tipping point' and deteriorates towards disease. An example of this is a recent study by the group of

Scheffer into 'critical transitions'. They describe how microbial communities in the human intestine influence the resilience of the intestinal ecosystem, and under certain circumstances can pass a tipping point and induce profound health implications.[35]

One more field of research that will become important in the future also needs to be mentioned. That is the interaction between quantitative facts and qualitative knowledge. As anthropologist Mol described, people do not relate to biomedical knowledge in such a way that they can experience it as a reality inside their body. In that sense, biomedical facts alienate people from the direct experience of health and disease.[36] A solution could be the use of a mixed methods approach, by combining quantitative facts with qualitative knowledge; for example, in a phenomenological description of a topic. The result of such an approach in practice was described by Mol *et al.* This concerned dieticians who noticed that clients could not connect to calories and nutrients and just got frustrated by their incompetence to change their lifestyle. Once an approach was used by which clients could connect to food products and experience what these did to their well-being, they could integrate also quantitative knowledge, as well as master and change their dietary habits.[37]

Such a combined approach may prove effective in closing the gap shown by our results between stakeholders with a university education and those without (Chapter 4). However, such a mixed methods approach would require a fair amount of further research in order to develop an equally sound body of qualitative knowledge, as the one available on quantitative knowledge.

NUTRITION and HEALTH

Our empirical study suggests that, even though conventional nutrient analyses of food products from various production systems, like often in literature, did not show systematic differences, differences in health effects could be found after consumption, even on genome level. With an increase in the diversity of food production methods in society – for example, in sterile high-tech methods with LED illumination – the question about health effects for the consumer becomes increasingly relevant. And although our study does not give a final proof regarding favourable health effects of organic food, our study did provide novel indications for potential health benefits that deserve further exploration. Indeed the combination of a lower body weight, higher immune titres on vaccinations, and in reaction to the challenge of a mimicked illness, a more 'alert' immune reaction, as well as a faster recovery from retarded growth, could be interpreted as health benefits.

As described, research on the health effects of foods from different production systems is complex.

Future research on this question should apply rigorous study designs and well-considered product choices of research organisms and effect parameters.

It is generally accepted that *intervention studies* on humans are most informative. However, these are expensive and would need a far sounder basis of physiological markers than is presently available, before delivering new and relevant information. More basic knowledge will first have to be elaborated in animals. A model that uses pigs would be a logical next step, after our study on chickens. However, such a study would also be quite costly.

From a pragmatic point of view, it would be useful to develop a research model with small organisms on which much fundamental research has already been performed. This would be relatively cheap and fast, and could provide fundamental information about physiological processes that might be influenced by organic foods. Such an approach could provide a basis for subsequent research in higher organisms.

Examples of such small organisms are C. Elegans, Drosophila and yeast. From these, the C. Elegans model is less suitable, as it feeds on bacteria. This would imply that bacteria should first be fed in different products, the juices of which have passed a bacteria filter to prevent contamination, before subsequently being fed to the worm. Although this would be feasible, we feel that this approach is too indirect. Using Drosophila, however, could provide a suitable model for such a feeding experiment, as it feeds on fresh products, is well-characterised for a variety of human functions and diseases,[38,39,40,41] and already has proven a sensitive model for measuring the physiological effects of foods from different agricultural systems.[42] As far as using yeast as a model for testing food product quality, to our knowledge, no research is currently available.

Another pragmatic and promising approach would be to include questions about the production source of food in *observational studies*. In the prospective KOALA study by Maastricht University and partners, supervised by Thijs and Dagnelie, which has been running since the year 2000, children who had been fed organic dairy products showed, at two years of age, a 33% lower incidence of eczema than those on a diet using conventional dairy products.[43] Once such an effect is found, more in-depth investigation about causations can be done, which in the above case revealed higher amounts of conjugated linoleic acid in organic dairy from cows fed relatively more grass and hay, which could also be found in the breast milk of the mothers.[44,45]

These results indicate the use of a combination of different approaches in research on the health effects of organic food.

About objections against organic nutrition and research about health effects

Although outside the scope of this thesis, it is important to at least briefly mention two often heard objections in connection to organic food.

One such objection to the research of health effects of organic food is that the overall diet, both in terms of nutrient composition and dietary pattern, is much more decisive than the production method of individual products could ever be. Here, observational studies present an outcome from yet another perspective, with an interesting interconnection. They show, as was recently again confirmed in a French study[46], that consumers of organic products choose significantly healthier lifestyles and diets than consumers of conventional products. Another recent study showed that consumers who eat organic products have an overall healthier lifestyle and diet than consumers on a diet including functional foods.[47] These results indicate that, first of all, it is not easy to research the isolated health effects of organic foods in observational studies, as consuming organic food and a healthier life style can be associated a priori. It would therefore be difficult, even when adjusting for overall diet composition, to completely exclude the influence of other lifestyle factors on health. Nevertheless, it could be speculated that, íf more health effects from organic products could be shown (e.g. in intervention studies), this might inspire more people to adopt an overall more healthy lifestyle, including the switch to organic food. Other research has indicated that a switch to 'organics' brings a gradual, personal 'shift of paradigm', whereby new visions and goals are developed.[48]

A second often expressed objection against organic nutrition is that organic production systems will never be able to provide sufficient amounts of food to feed the rapidly growing world population. However, in 1994, the Dutch Scientific Council for Government Policy (WRR) published a study[49] about the consequences of different perspectives on sustainability in relation to risks for the environment, economy and policy. Several areas were addressed, including energy, water supply, natural resources and also the world food supply, from the perspective of a strongly growing world population. For each area, different scenarios were discussed, and on the theme of world food supply, also organic agriculture was included. The conclusion in this report was that it would be possible to feed the world population using an organic type of production, but that the consumption of animal proteins should be reduced and there should be a fair distribution of food. It can be argued that the first point – a reduction in the consumption of animal foods – would also be favourable to the health of western populations, while the second point should be part of a general policy striving for world peace and stability.

Moreover, recently the Special UN Rapporteur on the right to food, Olivier De Schutter, stated in his report[50] that 'A new paradigm on well-being, resilience and sustainability must be designed to replace the productivist paradigm and thus better support the full realisation of the right to food. The equation is complex, but is one that can be solved'. De Schutter argues that the agro-ecological approach provides the best prospects to feed the world – including the developing world – from the perspective of climate change.

Although these two examples are by no means exhaustive, these reports from respected organisations do describe organic agriculture as a serious option.

Conclusion

This thesis started with the aim to elaborate an integral concept of health, in a modern and scientific way, connecting with the integrity of life as a whole, and offering a context for interpreting health research in a broad sense. We approached the topic of health from several angles, in both a conceptual and an analytical manner, and hope to have put forward elements that support the creation of more resilient and self-regulating systems in society (Figure 3).

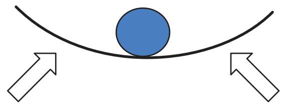

Figure 3 Visualisation of a resilient and self-regulating system, with a dynamic equilibrium

We formulated a new, dynamic concept of health, addressing patients as whole human beings and as more than just their illness. We elaborated this concept further into a broad range of indicators, covering 6 dimensions and 32 aspects, that connect health to 'life as a whole'. We conclude that, with maintaining the intentions of the WHO, it is possible to characterise and operationalise health in a way that connects to science, as well as to people's experience of daily life.

Furthermore, we conclude that, even though there is insufficient evidence on the health effects of organic food, our research results did provide new indications of health benefits, relating to the new health concept, which deserve further exploration. Future studies on this question should apply rigorous research designs, well-considered choices with respect to products, research organisms and effect parameters.

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Summary
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Summary

Health has always been an important value for human beings. But what is health? How is it defined? And how can one evaluate which influences are effective in strengthening health? This thesis addresses these questions in a concise way. It starts by pointing at some recent problems that underline the need to work on the topic of health. Subsequently, the research on this topic’ is presented in two separate sections. Part one deals with questions about the definition of health, from history up to the present time, leading to the proposal for a new concept of health and its evaluation. The second part discusses nutrition – a topic that is known to have a great influence on health. Here, a special focus is on the question of whether the way food is being produced would have an impact on the health of its consumer.
The thesis has an overarching aim to elaborate an integral concept of health, connected to the integrity of life, and offering a context for the interpretation of results in health research, from a broad perspective.

The two parts of the thesis:

PART I - HEALTH

In a concise, historical overview about the perception of health **(Chapter 2),** it is described how in ancient times health was perceived as a balance of qualities. For example, Hippocrates’ perception of health was a balance of four ‘humours’, which together constituted the human being; other cultures described different qualities, but also had the notion of health as a matter of balance and wholeness. Aristotle addressed the virtue of maintaining ‘the mean’ and described the virtue of personal development towards ‘eudaimonia’. For centuries, this perception continued to be the basis of the thinking on health and disease. Then, in the Renaissance and the age of Enlightenment, the physical basis of diseases were explored, resulting in health being viewed as the absence of disease. The connected analytical approach appeared very fruitful and today has resulted in a vast biomedical body of knowledge, with ongoing specialisations in the medical profession on ever more detailed levels.
With the establishment of the WHO in 1948, a very broad and idealistic definition of health was formulated, addressing the ultimate well-being of the human being on a physical, mental and social level. The definition was intended as a goal to work towards, for the happiness and well-being of the entire world population. This was a major step forward. However, health was described as a state of complete well-being, something that unintentionally promotes medicalisation. With the increase in chronic diseases, combined with the ongoing development of medical technology and diagnostic tools, this definition is becoming counterproductive. According to its static

formulation, nearly everyone is a patient in need of ongoing treatment, and it does not address people's resilience, the human capacity and potential for adapting to and coping with new situations through self-management.

We considered this to be a shortcoming, and **Chapter 3** describes how we intended to overcome this by formulating a new dynamic concept of health: 'Health as the ability to adapt and to self-manage, in the face of social, mental and physical challenges of life'. This concept, similar to the WHO's definition, describes the physical, mental and social domain of the human being, and thus has a broad and integral scope. The difference is that the concept emphasises the potential to be or become healthy, even when affected by disease, as well as the potential of personal growth and development towards fulfilment of personal aims in life. This concept is a beginning, a general characterisation of health, and does not claim to be exhaustive or to address all factors that contribute to such personal development.

The support for this concept was tested among different stakeholders in healthcare in the Netherlands **(Chapter 4),** and was well-received. Respondents described how they felt that the new concept addresses their strengths and their potential, instead of their weaknesses. However, they also warned that not all people could be expected to be capable of this 'ability to adapt and self-manage', at least not without appropriate guidance and support.

When exploring indicators of health in order to operationalise the concept, a large variety of elements was mentioned by the stakeholders. Elements were categorised into the following 6 main dimensions: bodily functions, mental functions & perception, the spiritual/existential dimension, quality of life, social & societal participation, and daily functioning; together, the dimensions contain 32 underlying aspects. These dimensions and aspects were tested, in a quantitative way, among approximately 2000 stakeholders. The outcome revealed large differences in interpretation with respect to the content of health. This ranged from a 'narrow', mainly biomedical interpretation, which was strongest among policymakers and researchers, to a 'broad' interpretation whereby all 6 dimensions were considered nearly equally important elements of the content of health. The latter opinion was shared by patients having a broad range of diseases. Between these two extremes were the scores of the health professionals. Looking at this group in more detail showed that nurses tended to mainly favour the broad perception, as did the patients, whereas the perception of physicians was more 'narrow' and biomedical.

Analyses showed that one of the most influencing factors in the thinking on health was the same for all stakeholders, namely that of having experienced disease themselves. Such an experience appeared to reduce the importance of the bodily aspects, in favour of a higher valuation of the spiritual/existential dimension, and a tendency towards a broader view on health. To avoid confusion between the bio-medical view of health being 'the absence of disease' and the broad interpretation of health, the concept of ‘positive health’ is proposed to represent the broad perspective, and is visualised in a web diagram with 6 axes representing the dimensions, which thus appear all equally important. A person can indicate his/her experienced level of functioning with respect to each of the dimensions and, if so desired, can search, individually or with support, for ways to improve their situation. In this way, an integral approach to health promotion can be further developed.

PART II – NUTRITION and HEALTH

Chapter 5 describes how the perception of nutrition, from ancient times up to the present, showed a development congruent with that of medicine. A paradigm shift occurred from the 16th century onwards, towards an analytical way of thinking, which also affected food production and resulted in modern industrial agriculture and food processing, in large parts of the world. Yet, a little over a century ago, a critical countermovement emerged of consumers, who were shortly after that joined by farmers, who emphasised that they considered human beings as well as food (both vegetable and animal) as living entities and more than merely ‘the sum of the parts’. An agricultural approach of ecological farming systems developed, with self-regulating properties aimed at balanced and resilient soil life, plants and animals. The hypothesis was that food products from such a source would strengthen the health of their consumers. Until recently, the two visions of agricultural production mainly collided in emotional debate, but modern analytical omics’ techniques may help to overcome the emotions and provide insight into the relationship between agricultural approaches and their impact on crops and animals. These techniques provide a way to address the notion that food products contain thousands of compounds, which shift in ‘clouds’ related to the production system, whereas within the physiology of the consuming organism these techniques also show a richness in patterns, which become influenced by the consumption of food. This modern development in the field of nutrition may enable a paradigm shift, similar to what might occur in the field of medicine, towards once again perceiving ‘wholes’ (whole products and organisms), but on a much higher level of knowledge than in ancient times.
This thesis describes how we attempted to connect research on the health effects of food to the new dynamic concept of health. To elaborate this approach, we chose to compare the possible health effects connected to two different food production systems, namely the conventional and the organic system. Organic food production can be considered as the currently most commonly used systems approach in animal and crop farming, which aims at maintaining the natural resistance of plants and animals, and excludes both pesticides and the preventive use of antibiotics. The question of possible beneficial effects related to the consumption of products from this production system is a topic of large societal interest, but also one of scientific controversy.

First, **Chapters 6 and 7** give an overview of the research on this topic. Different types of traditional nutritional research – analyses and consumption studies – are described, as well as the difficulties connected to the design of studies on this topic. On the basis of the available research, it was concluded that there are indications of a beneficial effect on health from organically produced food, but the differences in quality between organic products is large, and so is the influence of annual climatic conditions.

Chapter 8 describes how the 'ability to adapt' in the new concept of health can be expanded towards applying it in the research on health effects, such as in nutritional research. Different physiological systems are described that could be challenged and it is argued that the coherence in the recovery of various physiological processes and parameters towards homeostasis reflects a qualitatively good state of health.

Chapters 9 and 10 present a study in which this approach of applying a challenge to study health effects was used. In an animal study, using an immunological chicken model as a model for humans, the effects on health from feeds from either organic or conventional production were studied, in a fully controlled situation. An immunological challenge was applied in young animals of the second generation. This resulted in stunted growth with subsequent catch-up growth, and in strong immunological, physiological, metabolic and gene regulation parameters, with significantly different patterns between the two feed groups. The group on organic feed showed the stronger reaction, immunologically and metabolically, as well as a faster growth resumption. This pattern was described in a publication in 2010 as more 'resilient'; in fact, based on the previous chapters, it could be argued that this pattern could be called 'healthier'.

Chapter 10 describes the results of the genomics analyses of the gut of the animals. Although 49 genes were differentially regulated between the feed groups, at the time we could not interpret the phenomena. Nevertheless, it is an indication that, also on gene level, influences from feeds from different production systems can be found.

In **Chapter 11,** the General Discussion, several strengths and limitations of the presented studies are described, as well as their implications. Concerning the further elaboration of the health concept, a framework of different related topics is proposed, which would require further attention. In short, these topics are:

1. Differentiation between *positive health* and *the domain of curative medicine,* in order to give balanced attention to both, in a *two continua model* of health and disease, across the human lifespan.
2. In addition to the regular content of '*prevention*' – *the three Ps:* health *protection* against external risks, *prevention* of diseases and risky behaviour, and health *promotion* in the sense of promoting conventional healthy behaviour – also the promotion of *positive health* is added.
3. Concerning the *determinants* of health: differentiation between the level at which *individuals* can create health-improving environments for themselves, and the level where this is an overarching *public responsibility*. Concerning *individual*

determinants of health: differentiation between *external* and *internal resources* for health and the provision of *education* about these resources, for adults as well as in schools.

4. *Indicators/outcome measures* will be needed to assess levels of positive health and to evaluate developments. Differentiation between health on both an *individual* and a *population level,* and between indicators/outcome measures that are *self-reported and subjective* and those that are *externally acquired and objectivised.*
5. Concerning the *training of health professionals*: in addition to training in pathology and treatment, it is important to also include familiarity with a broad perspective on health, knowledge about positive psychology, training in personal resilience and self-management, as well as skills for shared decision-making and motivational interviewing.
6. *Research* remains important, whereby clinical research for *evidence-based medicine* needs to interact with *practice-based evidence* from clinical practice, in order to connect to the reality of the practice.

Concerning the topic of nutrition and health it is concluded that, overall, too little research has been done on this topic to draw firm conclusions other than that there are indications of beneficial health effects from organic nutrition. But although our animal study (**Chapter 9 and 10**) did not provide final proof of favourable health effects of organic food, the study did provide novel indications for potential health benefits that deserve further exploration. For the animals on organic feed, the combination of a lower body weight, higher immune titres on vaccinations, and in reaction to the challenge of a mimicked illness, a more 'alert' immune reaction, as well as a faster recovery from retarded growth, could be interpreted as health benefits. Research on the health effects of foods from different production systems is complex but not impossible. From a pragmatic point of view, it would be useful to develop a research model using small organisms on which much fundamental research has already been performed. This would be relatively cheap and fast, and could provide fundamental information about physiological processes that might be influenced by organic foods. Such an approach could provide a basis for subsequent research in higher organisms. It is proposed that, first, the Drosophila, the fruit fly, is used, as this has already proven to be a sensitive model for measuring the physiological effects of foods from different agricultural systems. In addition, it would be a pragmatic and promising approach to include questions about the production source of food in observational studies. An example is the prospective KOALA study by Maastricht University and partners, running since the year 2000, where children, who had been fed organic dairy products, at two years of age, showed a 33% lower incidence of eczema than those on a diet using conventional dairy products. Once such an effect is found, more in-depth investigations about the causes of this effect can be done.

Using a combination of different approaches in research on the question of health effects of foods from different production systems is the most promising, to find answers with respect to this topic of large societal interest.

Samenvatting

Gezondheid is voor de mens altijd een belangrijke waarde geweest. Maar wat is gezondheid precies? Hoe is het gedefinieerd? En hoe kan men beoordelen welke invloeden effectief zijn voor het bevorderen van de gezondheid? Dit proefschrift gaat over deze vragen. Allereerst wordt een aantal recente problemen beschreven, die de noodzaak onderstrepen om aan het onderwerp van de gezondheid te werken. Vervolgens wordt het onderzoek over dit onderwerp besproken, in twee afzonderlijke delen. Deel één betreft vragen over de definitie van gezondheid, van het verleden tot aan de huidige tijd, en leidt tot het voorstel voor een nieuw concept van gezondheid, en de evaluatie van dat concept. Deel twee behandelt het thema voeding - een onderwerp waarvan bekend is dat het een grote invloed heeft op gezondheid. De focus ligt hier op de vraag of de manier waarop voedsel wordt geproduceerd een invloed zou kunnen hebben op de gezondheid van de consument.
Dit proefschrift heeft als overkoepelende doelstelling een integraal concept van gezondheid uit te werken, dat verbonden is met de integriteit van het leven. Het biedt een context voor de interpretatie van resultaten in onderzoek rond gezondheid, vanuit een breed perspectief.

De twee delen van het proefschrift:

DEEL I - GEZONDHEID

In een beknopt historisch overzicht (**hoofdstuk 2**) over de visie op gezondheid, wordt beschreven hoe in de oudheid gezondheid werd gezien als een evenwicht van kwaliteiten. Zo is bijvoorbeeld Hippocrates' perceptie van gezondheid een balans van de vier 'humores', die tezamen de mens vormden; andere culturen beschreven andere kwaliteiten, maar hadden ook een visie op gezondheid als een toestand van evenwicht en heelheid. Aristoteles sprak van de deugd van het handhaven van 'het midden' en beschreef de deugd van persoonlijke ontwikkeling naar 'eudaimonia', nu wel vertaald als 'tot bloei komen'. Eeuwenlang vormde deze visie de basis van het denken over gezondheid en ziekte. Dat begon te veranderen vanaf de Renaissance en de Verlichting, toen de fysieke basis van ziekten verkend begon te worden, wat er tenslotte toe leidde dat gezondheid beschouwd werd als de afwezigheid van ziekte. De met deze benadering verbonden analytische aanpak bleek zeer vruchtbaar en heeft inmiddels geresulteerd in een zeer uitgebreide biomedische kennis, met nog steeds toenemende specialisaties, op steeds gedetailleerder niveau in de geneeskunde. Met de oprichting van de Wereld Gezondheid Organisatie, de WHO, in 1948, werd een zeer brede en idealistische definitie van gezondheid geformuleerd, die gezondheid omschrijft als 'een toestand van compleet welbevinden op fysiek,

mentaal en sociaal niveau, en niet alleen de afwezigheid van ziekte'. De definitie was bedoeld als doel om na te streven, ten dienste van het geluk en het welzijn van de gehele wereldbevolking. Dit was voor de mensheid een belangrijke stap voorwaarts. Echter, doordat gezondheid werd beschreven als een toestand van volledig welbevinden, werd onbedoeld medicalisering bevorderd, om dat ideaal maar te bereiken. Met de toename van chronische ziekten, in combinatie met de voortgaande ontwikkeling van de medische technologie en diagnostiek, wordt deze definitie contraproductief. Met de statische formulering van gezondheid als 'toestand', is vrijwel iedereen een patiënt die doorlopend behandeling behoeft, en wordt niet de veerkracht van mensen aangesproken en het menselijke vermogen om zich aan te passen en om te gaan met nieuwe situaties met behulp van zelfmanagement.
Wij beschouwden dit als een tekortkoming van de definitie van gezondheid en in **hoofdstuk 3** wordt beschreven hoe we probeerden dit probleem op te lossen door het formuleren van een nieuw, dynamisch concept van gezondheid: 'Gezondheid als het vermogen om je aan te passen en je eigen regie te voeren, in het licht van de sociale, mentale en fysieke uitdagingen van het leven'. Dit concept beschrijft, net als de WHO-definitie, het fysieke, mentale en sociale domein van de mens en heeft dus een brede en integrale scope. Het verschil is dat het concept de potentie benadrukt om gezond te zijn of te worden, zelfs wanneer er sprake is van een ziekte, en ook het vermogen van persoonlijke groei en ontwikkeling in de richting van het vervullen van persoonlijke doelen in leven. Dit concept is een begin, een algemene karakterisering van gezondheid, en heeft niet de pretentie volledig te zijn of alle factoren te benoemen die bijdragen aan deze persoonlijke ontwikkeling.
Het draagvlak voor dit concept werd onderzocht onder verschillende stakeholders in de gezondheidszorg in Nederland (**hoofdstuk 4**), en het werd positief ontvangen. De ondervraagden beschreven hoe zij ervaarden dat het nieuwe concept hen in hun kracht en hun mogelijkheden aanspreekt, in plaats van in hun zwakte. Maar men waarschuwde dat niet van alle mensen zonder meer verwacht kon worden dat zij tot dit 'vermogen om je aan te passen en je eigen regie te voeren' in staat zijn, althans niet zonder passende begeleiding en ondersteuning.
Bij een verkenning van de indicatoren van de gezondheid ('waar lees je gezondheid aan af'), om het concept te operationaliseren, werd door de stakeholders een grote verscheidenheid aan elementen genoemd. Deze werden ingedeeld in zes hoofddimensies: lichamelijke functies, mentale functies en beleving, de spirituele/existentiële dimensie, kwaliteit van leven, sociaal-maatschappelijke participatie en dagelijks functioneren; met daarbij 32 onderliggende aspecten. Deze dimensies en aspecten werden vervolgens op een kwantitatieve manier getest onder bijna 2000 stakeholders. Het resultaat toonde grote verschillen in interpretatie, wat betreft de inhoud van gezondheid. Dit varieerde van een 'smalle', voornamelijk biomedische interpretatie, vooral onder beleidsmakers en onderzoekers, tot een 'brede' interpretatie

waarbij alle zes dimensies als bijna even belangrijke onderdelen van gezondheid werden beschouwd. De laatstgenoemde visie werd gedeeld door patiënten met een breed palet aan ziekten. De oordelen van de behandelaren lagen tussen deze twee uitersten. Bij een nadere beschouwing van deze groep bleek dat verpleegkundigen neigden naar een brede visie op gezondheid, net als de patiënten, terwijl artsen neigden naar een 'smalle' en biomedische kijk.
Analyses toonden aan dat een van de meest invloedrijke factoren bij het denken over gezondheid voor alle stakeholders gold, namelijk het zelf hebben doorgemaakt van ziekte. Deze ervaring bleek de waarde die gehecht werd aan lichamelijke aspecten te verminderen, terwijl de spirituele/existentiële dimensie hoger gewaardeerd werd en een tendens naar een bredere kijk op de gezondheid optrad. Om verwarring te vermijden tussen de biomedische visie op de gezondheid als 'de afwezigheid van ziekte' en de brede interpretatie van de gezondheid, wordt het concept van de 'positieve gezondheid' voorgesteld voor het brede perspectief, gevisualiseerd in een web-diagram met zes assen voor de dimensies, die daarmee allemaal als even belangrijk verschijnen. Iemand kan in dit diagram het zelfervaren niveau van functioneren op de verschillende dimensies aangeven en dan desgewenst, alleen of met hulp, zoeken naar manieren om de eigen situatie te verbeteren. Op deze manier kan een integrale aanpak van gezondheidsbevordering verder worden ontwikkeld.

DEEL II - VOEDING en GEZONDHEID

Hoofdstuk 5 beschrijft hoe het denken over voeding, vanaf de oudheid tot heden, een ontwikkeling vertoont die vergelijkbaar is met die in de geneeskunde. Vanaf de 16e eeuw vond er een paradigmaverschuiving plaats naar een analytische manier van denken, die ook voedselproductie beïnvloedde en leidde tot de moderne industriële landbouw en de voedselverwerkende industrie, in grote delen van de wereld. Iets meer dan een eeuw geleden ontstond echter een kritische tegenbeweging van consumenten, waar zich spoedig ook boeren bij aansloten, die benadrukten dat zij de mens, maar ook plantaardig en dierlijk voedsel, beschouwden als levende wezens en als meer dan alleen 'de som van de delen'. Er ontwikkelden zich uit die stroming ecologische landbouwsystemen, die een versterking van zelfregulerende eigenschappen beoogden, met als resultaat een evenwichtig en veerkrachtig leven van bodem, planten en dieren. De hypothese was dat voeding uit een dergelijke productiemethode de gezondheid van de consument zou versterken.
Tot voor kort botsten deze twee benaderingen in de landbouw in een vooral emotioneel debat, maar moderne analytische technieken zoals de 'omics methoden' kunnen helpen om vanuit feiten te redeneren en inzicht te geven in de relatie tussen productiemethoden en hun invloed op gewassen en dieren. Met behulp van deze

technieken wordt zichtbaar dat voedingsmiddelen duizenden verbindingen bevatten, die in 'wolken' verschuiven in samenhang met de productiemethode. Ook de fysiologie van de consument kan met deze technieken bestudeerd worden en daarmee wordt een rijkdom aan patronen zichtbaar, die beïnvloed worden door de consumptie van voedsel. Deze moderne technieken zouden op het gebied van voeding, evenals op het gebied van de geneeskunde, een paradigmashift mogelijk kunnen maken, naar een hernieuwde visie op samenhangen en gehelen (het product en organismen als een geheel). Maar dan op een veel hoger niveau van kennis, dan in oude tijden.
Dit proefschrift beschrijft hoe we het onderzoek naar gezondheidseffecten van voeding, hebben verbonden met het nieuwe, dynamische concept van gezondheid. Om deze benadering uit te werken hebben we gekozen voor het vergelijken van effecten op gezondheid van twee verschillende productiesystemen van voedsel, namelijk het gangbare en het biologische productiesysteem. De biologische voedselproductie is op dit moment het meest gebruikte systeem dat er naar streeft om de natuurlijke weerstand van planten en dieren te behouden en zowel pesticiden, als het preventief gebruik van antibiotica, uitsluit. Het vraagstuk van het mogelijk gunstige effect van de consumptie van biologische producten, is een onderwerp van groot maatschappelijk belang, maar ook een van wetenschappelijke controverse.
De **hoofdstukken 6 en 7** geven een overzicht van het onderzoek over dit onderwerp. Verschillende soorten traditionele voedingsonderzoeken - stofanalyses en consumptie studies - worden beschreven, evenals de moeilijkheden die verbonden zijn met het ontwerpen van onderzoek op dit gebied. Op basis van het beschikbare onderzoek werd geconcludeerd dat er aanwijzingen zijn voor een gunstig effect van biologisch geproduceerd voedsel op de gezondheid, maar de verschillen in kwaliteit tussen biologische producten is groot, evenals de invloed van de jaarlijkse klimatologische omstandigheden.
Hoofdstuk 8 beschrijft hoe het 'vermogen je aan te passen' in het nieuwe concept van gezondheid kan worden toegepast in het voedingsonderzoek naar effecten op de gezondheid. Verschillende fysiologische systemen worden beschreven die kunnen worden uitgedaagd middels een ‘challenge’ en er wordt gesteld dat de coherentie in het herstel naar homeostase, van de verschillende fysiologische processen en parameters, staat voor een kwalitatief goede staat van gezondheid.
De **hoofdstukken 9 en 10** beschrijven een studie waarin dit toepassen van een ‘challenge’ voor het bestuderen de gezondheidseffecten, werd gebruikt. In een studie bij dieren, met een immunologisch ‘kippenmodel’ als model voor de mens, werden onder volledig gecontroleerde omstandigheden de gezondheidseffecten bestudeerd van producten uit de biologische of conventionele voedselproductie. Een immunologische ‘challenge’ werd toegepast bij jonge dieren van de tweede generatie; zij werden ‘ziek’ gemaakt. Dit resulteerde in een remming van de groei, met na een periode (de ziekte), een inhaalgroei. In de verschillend gevoede groepen bleken aanzienlijk

verschillende reactiepatronen in de parameters voor immunologische en fysiologische processen en de genregulatie. De groep op de biologische diervoeders toonde een sterkere reactie, immunologisch en fysiologisch, en evenals een snellere inhaalgroei. Dit reactie en herstel-patroon werd in een publicatie over dit onderzoek in 2010 beschreven als meer 'veerkrachtig'; in feite, op basis van de voorgaande hoofdstukken, kan nu worden gesteld dat dit patroon 'gezonder' genoemd zou kunnen worden.

Hoofdstuk 10 beschrijft de resultaten van de genomics (gen-)analyses van de darm van de dieren. Hoewel 49 genen bij de voergroepen verschillend waren gereguleerd, konden we op dat moment de verschijnselen niet interpreteren. Niettemin is het een aanwijzing dat ook op gen-niveau, invloeden van voeders van verschillende productiesystemen te vinden zijn.

In **hoofdstuk 11**, de algemene discussie, worden verschillende sterktes en beperkingen van de gepresenteerde onderzoeken beschreven, evenals de betekenis ervan. Wat de verdere uitwerking van het gezondheidsconcept betreft, wordt een raamwerk voorgesteld van verschillende verwante onderwerpen, die nadere aandacht verdienen. In het kort zijn deze onderwerpen:

1. Differentieer tussen positieve gezondheid en het domein van curatieve gezondheidszorg, teneinde evenwichtig aandacht te kunnen schenken aan beide. Een 'twee continua model' van gezondheid en ziekte, gedurende het mensenleven, wordt voorgesteld.
2. In aanvulling op de gebruikelijke inhoud van 'preventie' - de drie P's: bescherming (*protection*) van de gezondheid tegen externe risico's, *preventie* van ziekten en risicovol gedrag, en de bevordering (*promotion*) van de gezondheid in de zin van het bevorderen van conventionele gezond gedrag – wordt ook de bevordering van positieve gezondheid voorgesteld.
3. Met betrekking tot de determinanten van gezondheid (die gezondheid bevorderen): differentieer tussen het niveau waarop individuen een gezondheid bevorderende omgeving kunnen creëren voor zichzelf, en het niveau waar dit een overkoepelende publieke verantwoordelijkheid is. Met betrekking tot de individuele determinanten van gezondheid: maak onderscheid tussen externe en interne middelen voor de gezondheid en het verstrekken van voorlichting over deze middelen, voor zowel volwassenen als op scholen.
4. Indicatoren/uitkomstmaten zullen nodig zijn om het niveau van positieve gezondheid te beoordelen en om de ontwikkelingen te kunnen evalueren. Differentieer tussen gezondheid op zowel individueel als op populatieniveau, en tussen de zelfgerapporteerde en subjectieve indicatoren/uitkomstmaten en die uitkomstmaten die objectieve gegevens verschaffen.
5. Met betrekking tot de opleiding van gezondheidswerkers: in aanvulling op opleiding in de pathologie en de behandeling, is het belangrijk om ook de vertrouwdheid met een brede kijk op de gezondheid, de kennis over de positieve

psychologie, training in persoonlijke veerkracht en zelfmanagement, evenals vaardigheden voor gedeelde besluitvorming en 'motivational interviewing' op te nemen.
6. Onderzoek blijft belangrijk, waarbij het klinisch onderzoek ten behoeve van evidence-based medicine gecombineerd moet worden met practice-based-evidence, afkomstig uit de klinische ervaring, om een verbinding te blijven maken met de realiteit van de praktijk.

In relatie tot het onderwerp voeding en gezondheid wordt geconcludeerd dat in het algemeen te weinig onderzoek is gedaan naar het hier beschreven onderwerp, om meer te kunnen constateren dan dat er aanwijzingen zijn voor gunstige gezondheidseffecten van biologische voeding. Maar hoewel de dierstudie (**hoofdstuk 9 en 10**) geen definitief bewijs leverde voor gunstige gezondheidseffecten van biologische voeding, toonde de studie wel nieuwe indicatoren aan voor potentiële gezondheidsvoordelen, die verder onderzoek verdienen. Bij de dieren op het biologische voer kan de combinatie van een lager lichaamsgewicht, hogere immuun titers na vaccinaties, en in reactie op de 'challenge', een 'alertere' immuunreactie en een sneller herstel van vertraagde groei, als een voordeel voor de gezondheid worden geïnterpreteerd:
Onderzoek naar de gezondheidseffecten van voedingsmiddelen uit verschillende productiesystemen is complex, maar niet onmogelijk. Vanuit een pragmatisch oogpunt is het nuttig om een onderzoeksmodel te ontwikkelen met behulp van kleine organismen, waarop al veel fundamenteel onderzoek is uitgevoerd. Dit zou relatief goedkoop en snel kunnen, en dit kan fundamentele informatie geven over de fysiologische processen die kunnen worden beïnvloed door biologische voeding. Zo'n benadering kan een basis vormen voor verder onderzoek bij hogere organismen. Er wordt voorgesteld dat dit model te ontwikkelen met de Drosophila, de fruitvlieg, aangezien dit diertje zich reeds heeft bewezen als een gevoelige model voor het meten van de fysiologische effecten van voedingsmiddelen afkomstig van verschillende productiesystemen. Daarnaast is het een pragmatische en veelbelovende aanpak, om vragen over de productiebron van voedsel in observationele studies mee te nemen. Een voorbeeld daarvan is het prospectieve KOALA-onderzoek van de Universiteit van Maastricht en partners, dat sinds het jaar 2000 loopt en waarbij kinderen, die waren gevoed met biologische zuivelproducten, op de leeftijd van twee jaar een 33% lager voorkomen van eczeem toonden dan kinderen op een voedingspatroon met gangbare zuivelproducten. Zodra een dergelijk effect wordt gevonden, kunnen meer diepgaande onderzoeken worden toegevoegd, naar de oorzaken van dit effect.
Een combinatie van verschillende benaderingen, in het onderzoek naar de vraag van de gezondheidseffecten van voedingsmiddelen uit verschillende productiesystemen, is het meest veelbelovend, om antwoorden te vinden met betrekking tot dit onderwerp, dat een groot maatschappelijk belang heeft.

Valorisatie

Een moderne eis van de Universiteit Maastricht is om aan een proefschrift een beschouwing toe te voegen over de mogelijke valorisatie van het beschreven onderzoek. De definitie van valorisatie is "het proces van waarde creatie uit kennis, door kennis geschikt en/of beschikbaar te maken voor maatschappelijke (en/of economische) benutting en geschikt te maken voor vertaling in concurrerende producten, diensten en nieuwe bedrijvigheid"(naar de Landelijke Commissie Valorisatie 2011:8).
Een vijftal vragen worden hierbij meegegeven, nl. 1) de maatschappelijke en/of economische relevantie van het onderzoek; 2) doelgroepen buiten de wetenschap voor wie de resultaten interessant zijn; 3) concrete activiteiten en producten waarin de onderzoeksresultaten vertaald worden en vorm krijgen; 4) in hoeverre zijn deze activiteiten innovatief ten opzichte van bestaand aanbod?; 5) hoe krijgt dit traject vorm en wat is de planning?
Hieronder zal op deze vragen worden ingegaan, allereerst m.b.t. het thema gezondheid.

1) De maatschappelijke en/of economische relevantie.

Sedert de publicatie van het nieuwe, dynamische concept van gezondheid 'Health as the ability to adapt and to self manage' in de British Medical Journal in 2011, heeft het concept nationaal en internationaal veel weerklank gevonden. Vanuit de hele wereld werden waarderende reacties ontvangen, waaronder van de prominente public health professor Ilona Kickbush (Director of the Global Health Programme at the Graduate Institute of International and Development Studies, Geneva, Switzerland), die schreef "Great work guys! You have my support!".
De Nederlandse overheidsinstanties citeren het concept sindsdien in diverse documenten. Enkele voorbeelden:

- In het Actieplan van de 'Taskforce Life Sciences & Health - EU Connect' van de Topsector LSH, waarin wordt geschetst hoe Nederland zich kan profileren in Brussel in het kader van Horizon 2020,[1,2] wordt het nieuwe gezondheidsconcept in het hoofdstuk 'Waar staat Nederland voor', beschreven als het overkoepelende Unique Selling Point (USP) voor gezondheid, waaronder het dan voor vier thema's worden uitgewerkt.
- Minister Edith Schippers van min. VWS, installeerde in 2012 een Commissie Innovatie Zorgberoepen en Opleidingen binnen het Zorginstituut Nederland,[3] met als opdracht een nieuwe beroepenstructuur voor de zorg in 2030 te ontwerpen. Deze Commissie hanteert het nieuwe gezondheidsconcept als één van haar drie uitgangspunten.
- In een onderzoek dat het Verweij-Jonker Instituut in opdracht van bovengenoemde Commissie uitvoerde om het draagvlak voor het gezondheidsconcept te toetsen, bleek dit draagvlak groot te zijn.[4]

- In de VTV van 2014 van het RIVM[5] wordt aandacht besteed aan het nieuwe gezondheidsconcept en de uitwerking ervan.
- In een subsidiecall van NWO in 2013 met als titel 'Kwaliteit van leven en gezondheid',[6] werd het nieuwe gezondheidsconcept als karakterisering voor 'gezondheid' gehanteerd.

Ook het vervolgonderzoek waarin het concept werd getoetst en waarin een stap naar operationalisering werd gezet met het begrip 'positieve gezondheid', heeft veel aandacht getrokken. Onder 2) wordt hierover meer beschreven.
ZonMw kende in 2012 een ZonMw Parel toe aan de auteur van dit proefschrift, voor het werk aan het gezondheidsconcept, als uitdrukking van het feit dat dit werk als relevant wordt beschouwd.[7]

2) Doelgroepen buiten de wetenschap voor wie de resultaten interessant zijn.
Het begrip 'positieve gezondheid' omvat 6 dimensies die, zoals patiënten het omschrijven, "over het hele leven gaan". Werkers in de zorg typeren 'positieve gezondheid' als een integratie van het zorg- en het sociale domein en de focus op versterken van veerkracht en eigen regie spreekt aan. Juist de breedheid van het concept wordt door veel instanties gezien als een passend antwoord op en de transities en decentralisaties die anno 2014 en 2015 gaande zijn in de zorg. Het concept vormt dan een oriëntatie en een kader, dat men wil gebruiken bij de noodzakelijke herinrichtingen van de zorg. Enkele voorbeelden:
- GGD Nederland adopteerde het begrip 'positieve gezondheid' en wil het integreren op het gebied van het 'meten' in de Nationale Volksgezondheidsmonitor, op het gebied van de 'praktijk' d.w.z. bij het aanbod van handelingsperspectieven, en op het gebied van 'beleid'. Veel regionale GGD 's hebben 'positieve gezondheid' in hun beleidspannen opgenomen.
- De regio 'Noordelijke Maasvallei, bestaande uit een aantal gemeenten, waarbinnen zich een ziekenhuis, een GGZ-instelling, zorggroepen, sociale wijkteams, etc. bevinden, wil als hele regio binnen 5 jaar de medische en sociale zorg inrichten op basis van de inhoud van het concept 'positieve gezondheid'. Dat wil zeggen dat het versterken van de veerkracht en zelfredzaamheid van burgers veel aandacht zal krijgen.

  Andere regio's, met een vergelijkbare diversiteit van instellingen, volgen de ontwikkelingen in de Noordelijke Maasvallei met grote belangstelling.
- Thuiszorgorganisatie Emile wil zorg aanbieden waarin 'positieve gezondheid' geïntegreerd is.

NB. GGD Nederland, de Noordelijke Maasvallei en Emile Thuiszorg dienden ieder in 2014 een Pledge in bij 'Alles is Gezondheid' van VWS, waarin zij hun intenties met 'positieve gezondheid' verwoordden. [8]

- MVO Nederland organiseerde in 2013 en 2014 de MVO Expeditie Duurzame Gezondheidszorg, waarin 10 Nederlandse ziekenhuizen deelnemen. Het begrip 'positieve gezondheid' is hierbij een sleutelbegrip.
- Diverse organisaties werkzaam in het domein van 'werk en gezondheid' hebben het voornemen uitgesproken 'positieve gezondheid' op te nemen in hun beleid en werk.
- Stichting Zelfhulpgroepen heeft verklaard met 'positieve gezondheid' aan de slag te willen in hun groepen.[9]
- Zorgverzekeraar VGZ citeert 'positieve gezondheid' in diverse publicaties als een benadering die hen aanspreekt.

Diverse tijdschriften besteedden aandacht aan het gezondheidsconcept en de uitwerking, met interviews in o.a. Medisch Contact, TVZ-Tijdschrift voor Verpleegkundig Experts, GezondNu en Geron-Tijdschrift over ouder worden & samenleving.[10,11]
Tientallen lezingen werden door de auteur van dit proefschrift op verzoek gegeven over het thema, voor zorginstellingen, artsenorganisaties, eerstelijns zorgroepen, verpleegkundigen, verloskundigen, gehandicapten zorg, GGD-en en gemeentes, verzekeraars, etc.

3) Concrete activiteiten en producten waarin de onderzoeksresultaten vertaald worden en vorm krijgen.

Enkele voorbeelden, in relatie tot de hierboven genoemde doelgroepen:
- Vanuit GGD Nederland zijn op de drie terreinen: 'meten', 'praktijk' en 'beleid', werkgroepen gestart, waarin de auteur van dit proefschrift participeert. De werkgroep 'meten' met GGD-epidemiologen onderzocht de mogelijkheden om tot gevalideerde vragen voor 'positieve gezondheid' te komen en formuleerde een plan om in een aantal fases tot een meetinstrument te komen, dat enerzijds bruikbaar kan zijn voor de Nationale Volksgezondheidsmonitor en anderzijds geschikt is voor individueel gebruik, of binnen een consult met een hulpverlener.
- Het streven is de deelaspecten van het meetinstrument te verbinden met gevalideerde praktijkinterventies, zodat, indien iemand zijn/haar situatie op een bepaald aspect wil verbeteren, daarvoor suggesties beschikbaar zijn. Hieraan werkt o.a. de GGD-werkgroep 'praktijk' (zie hierboven).
- In de Noordelijke Maasvallei is een Werkgroep Positieve gezondheid actief om een implementatieplan te formuleren. ZonMw gaf een VIMP, een 'Verspreidings- en Implementatie-subsidie', om het proces aldaar te beschrijven.

- Met Emile Thuiszorg wordt een experiment gedaan, waarbij in een pilot-setting medewerkers worden getraind om met 'positieve gezondheid' aan het werk te gaan. Vervolgens worden de ervaringen van werkers en cliënten geëvalueerd.

4) In hoeverre zijn deze innovatief ten opzichte van bestaand aanbod?
De diverse instanties die met het nieuwe gezondheidsconcept en met 'positieve gezondheid' in zee gaan, verwoorden dat voor hen innovatief is om het zorg- en het sociale domein te integreren en daarmee scheidingen te slechten en meer te gaan samenwerken en tevens om naar de (veer)kracht van mensen te kijken en niet alleen naar de ziekten. Met de vigerende transities komt het concept precies op het goede moment, zo wordt uitgesproken.

5) Hoe krijgt dit traject vorm en wat is de planning?
Nu 'positieve gezondheid' met zoveel enthousiasme ontvangen wordt, is het van belang dat de kwaliteit van de inhoud en van de verdere uitwerking bewaakt wordt. De huidige invulling van het begrip is getypeerd door 6 dimensies, hetgeen essentieel is. Een stichting is in oprichting, die ten doel heeft deze kwaliteit te bewaken.

Over de valorisatievragen valt m.b.t. het thema voeding, zoals beschreven in dit proefschrift, het volgende te zeggen:

1) De maatschappelijke en/of economische relevantie.
De organisatie TNO Kwaliteit van Leven, in de vestiging in Zeist, werkt op dit moment al met challenges om gezondheidseffecten van voedsel te meten, met het begrip 'phenotypic flexibity' als gezondheidsnorm, en verwijst nu naar het nieuwe gezondheidsconcept als het kader waarbinnen deze benadering past, zo is te vinden in de TNO-brochure van het project 'Phenflex'.[12]

Indien de wetenschap zich meer zou gaan richten op de invloed van de teeltomstandigheden op de gezondheidswaarde van gehele producten, zoals beschreven in dit proefschrift, en de resultaten daarvan zouden worden doorgevoerd in de Nederlandse voedselproductie, zou deze er een onderscheidende dimensie bij kunnen krijgen, die commercieel interessant kan zijn voor de telers.

2) Doelgroepen buiten de wetenschap voor wie de resultaten interessant zijn.
De 'Agri-bussiness' is één van de grote pijlers van de Nederlandse economie en is vooral op export gericht. De omvang van deze agrarische export is zodanig, dat Nederland inmiddels de tweede positie heeft op de ranglijst van voedsel exporterende landen, na de VS. Dit is evenwel ook een kwetsbare sector, waar overproductie dreigt, en veel productiebedrijven maken moeilijke tijden door. De recente Russische boycot

van Nederlandse landbouwproducten maakt dat extra zichtbaar. Indien de relatie teeltomstandigheden en gezondheidswaarde meer aandacht krijgt, kan dit gunstige effecten hebben op de export .
Ook veel Nederlandse consumenten hebben overigens grote belangstelling voor dit thema.

3) Concrete activiteiten en producten waarin de onderzoeksresultaten vertaald worden en vorm krijgen.

Het is van belang dat dit thema wordt opgenomen in onderzoek agenda's, hetgeen nog niet het geval is.

4) In hoeverre zijn deze innovatief ten opzichte van bestaand aanbod?

De benadering, zoals beschreven in dit proefschrift is innovatief, omdat het een relatie beschrijft die nauwelijks onderzocht is maar wel potentie heeft, zoals werd beschreven.

5) Hoe krijgt dit traject vorm en wat is de planning?

Er zijn gesprekken gaande door de auteur van dit proefschrift om mogelijkheden voor verder onderzoek te onderzoeken.

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Dankwoord

'Life is what happens when you're planning other things', zie John Lennon, en zo verging het mij ook met mijn promotietraject. Ik had het idee om ooit te promoveren al lang uit mijn hoofd gezet – nooit een echte match tussen mij, een hoogleraar en een onderwerp – tót mijn eerste gesprek met André Knottnerus, in 2008. Ik was naar Maastricht gereisd om mijn zorgen te bespreken over het ontbreken van een werkbaar begrip van 'gezondheid'. Dat begrip leek te 'exploderen', met allerlei verschillende deelinvullingen door verschillende laboratoria. Was het niet aan huisartsen – en zo voelde ik mij nog steeds – om deze veelheid aan invullingen te integreren tot een geheel?, dat was mijn vraag. Het gesprek werd voor mij een inspirerende herkenning van een integrerend denker, deze hoogleraar huisartsgeneeskunde! André herkende het probleem en stelde voor dat ik met Henk Smid van ZonMw zou gaan praten. Ook Henk Smid herkende het probleem met het begrip gezondheid en stelde voor dat ik een Invitational Conference zou organiseren, i.s.m. met ZonMw en de Gezondheidsraad, met André in zijn rol als voorzitter. Dat werd het begin van een heel vruchtbare en vreugdevolle samenwerking over het thema gezondheid. Henk, ik ben je nog steeds heel dankbaar voor je voorstel, het initiatief voor deze conferentie!
Al op de terugweg van mijn eerste bezoek aan André dacht ik 'bij hem wel zou willen promoveren'. Ik besloot echter eerst de uitdaging van de conferentie aan te gaan. Tot mijn grote vreugde reageerde André daarna positief op mijn verzoek om bij hem te mogen promoveren. In mijn proefschrift wilde ik graag ook mijn eerdere onderzoek naar de gezondheidseffecten van voeding opnemen. Daarom zocht ik ook bij dat thema een promotor. Pieter Dagnelie kende ik al jaren, uit het gemeenschappelijke voedingsonderzoek in de KOALA-studie en een adviesraad. Het was een genoegen om Pieter in zijn hoogleraarsfunctie te vragen of hij deze rol voor mij zou willen vervullen en tot mijn vreugde stemde ook hij in.
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In dit proefschrift zijn onder meer twee empirische studies beschreven, die ik met diverse mensen heb uitgevoerd. In beide studies had ik een speciaal maatje, met wie ik intensief samenwerkte. Het is mij een vreugde dat zij beiden nu mijn paranimfen willen zijn! Dré Nierop, met jou werkte ik samen in het grote onderzoek met de kippen, en Marja van Vliet, met jou in het recente onderzoek naar de uitwerking van het gezondheidsconcept. Ik heb heerlijk met jullie beiden samengewerkt en dank jullie daar beiden zeer voor. Ook mijn reserve-paranimf Inge Boers, voor het geval Marja vanwege haar zwangerschap niet aanwezig kan zijn, ben ik heel dankbaar dat je deze rol op je wil nemen! Nu werken wij samen aan het verder implementeren van het gezondheidsconcept, wat ik met veel plezier doe!
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About the author

Machteld Huber was born in Apeldoorn, on 9 December 1951, and grew up in the rural area of Beekbergen. After completing the Royal HBS-B in Apeldoorn in 1969, she spent one year abroad, in London and Paris, before starting her studies in medicine at Utrecht University in 1970. During her medical studies, she spent one year in the United States and Canada, where she worked as a student intern in internal medicine and paediatrics. In 1977, she obtained her medical degree, as well as the similar American ECFMG Certificate. Wanting to connect with the roots of her profession, she started preparing for work in the tropics by practicing during 2.5 years as an intern in internal medicine, gynaecology and surgery in the Lukas Ziekenhuis in Apeldoorn and the Sophia Ziekenhuis in Zwolle. When, at the end of this period, she was introduced to medical philosophy and recognised it as also a possibility to fulfil her aim. Therefore, she decided to stay in the Netherlands and to study philosophy instead – in which she obtained her bachelor's degree in 1981. She then started her training as a General Practitioner at the VU in Amsterdam and became a registered GP in 1983. At the end of this training, she developed an illness, followed by three more different illnesses over the next three years. Whenever possible, she performed Locum General Practitioner work. This experience with illness had such an impact that she decided in 1986 to switch careers. She started to work, part time with drug addicts and as a researcher at the multidisciplinary Louis Bolk Institute in Driebergen, where she is also currently employed. In 1989, she stopped the work with drug addicts, and was asked to initiate an information centre on organic nutrition (Voedingsinstituut Dúnamis), where she filled the position of Director during 10 years, up to the year 2000, after which she went into full-time research work. However, as she missed patient contact, in 2004 she started part-time work as an intern in psychiatry at the Sinai Centre in Amersfoort, for people with war traumas. In 2005, she had to terminate this part-time work, when the Ministry of Agriculture, Nature and Food Quality granted the application for a big nutritional intervention study in chicken on biomarkers for health effects from organic versus conventional feed, which required her full attention. In this multicentre study with the Dutch institutes TNO, WUR and RIKILT, Machteld became project leader, as she had posed the question and, because of her work at the Louis Bolk Institute, was acquainted with agricultural topics. This study finished in 2008 with clear results, yet no conclusions could be drawn due to the lack of an operational definition of health. Machteld then decided to make 'health' her topic and addressed the Health Council of the Netherlands and the Netherlands Organisation for Health Research and Development, who also recognised the need for an operational definition of health. She was asked to organise an international invitational conference, which led to the new concept of health, as published in the BMJ. Following this, she performed a study to further operationalise the concept, which resulted in the broad concept of ‘positive health’. In 2012, for her work on the topic of health, Machteld received a ‘ZonMw Pearl’ award by the Netherlands Organisation for Health Research and Development. After obtaining her PhD, Machteld intends to further operationalise the concept of health for society.

Machteld is married to Guus van der Bie.

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