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Pruimboom, Leo

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Chapter 1. **Chronic systemic low-grade inflammation**


**The selfish immune system**

**When the Immune System Overrides the ‘Selfish’ Brain**

Leo Pruimboom\(^{1,2}\), Charles L. Raison\(^3\), Frits A.J. Muskiet\(^1\)

\(^1\)Laboratory Medicine, University Medical Center Groningen (UMCG), University of Groningen, P.O. Box 30.001 Groningen, The Netherlands;
\(^2\)Natura Foundation, Edisonstraat 66, 3281 NC Numansdorp, The Netherlands;
\(^3\)School of Human Ecology and School of Medicine and Public Health, University of Wisconsin Madison, Madison, WI, USA

**Corresponding author**

L. Pruimboom
Burgemeester Marijnenlaan 98
2585 DR Den Haag, The Netherlands
Email: cpni.pruimboom@icloud.com
Review

The Selfish Immune System

When the Immune System Overrides the ‘Selfish’ Brain

Leo Pruimboom\textsuperscript{1,2,*}, Charles L Raison\textsuperscript{3}, Frits A.J. Muskiet\textsuperscript{1}

1. Laboratory Medicine, University Medical Center Groningen (UMCG), University of Groningen, P.O. Box 30.001 Groningen, the Netherlands; f.a.j.muskiet@umcg.nl
2. Natura Foundation, Edisonstraat 66, 3281 NC Numansdorp, the Netherlands;
3. School of Human Ecology and School of Medicine and Public Health, University of Wisconsin Madison, Madison, WI, USA; raison@wisc.edu

* Correspondence: cpln.pruimboom@icloud.com

Abstract

The brain and the immune system are the only two systems of the human body that can dominate all others by extracting resources, including glucose. The brain dominates during daytime hours and stressful situations, whereas the immune system protects us principally at night, during periods of infection and when wounds are healing. Both systems are similarly capable of drawing on energy and other essential resources using strategies beneficial to their own function and anatomy. Human evolution has made the brain the most important of the body’s systems, resulting in a shift from strong to smart. However, the immune system is very old and robust; when necessary it is activated by a variety of non-specific immune challenges such as psychoemotional stress and most often when immune activating risk factors (including endotoxemia) are not solved in an appropriate timeframe. When chronically activated, the immune system demonstrates even more selfish behaviour than the selfish brain, inducing chronic low-grade inflammation and multiple related diseases. But before castigating the immune system for this behaviour, it is crucial to recognise that it is only doing what it is made for: trying to protect us.

Keywords

immune system; selfish brain; inflammation; evolution; stress; chronic disease; Alzheimer; fibromyalgia syndrome; insulin resistance
1. Introduction

1.1. The selfish brain and immune system in evolution

Low-grade inflammation; the cause of causes

Chronic inflammatory diseases (CID) are increasing in frequency, while treatments for these conditions are still in their infancy (1). Many of these diseases hardly existed 200 years ago (2) and proximate interventions addressing their genuine aetiologies have not been very successful (3). Chronic inflammation involves the innate and adaptive immune system (4), which can be considered very costly at the level of the use of resources, including energy (5), proteins and certain minerals, such as calcium (6) and magnesium (7-9). Long-term activation of the innate and adaptive immune system causes further maturation of antigen presenting cells (10). It is therefore plausible that low-grade inflammation is a direct cause of multiple diseases related with increased activity of the innate and adapted immune system including multiple autoimmune disorders and cancer. A recent meta analysis showed a linear association between C reactive protein (CRP), a marker for low grade inflammation and risk for breast cancer (11).

Physiological processes in all living organisms are direct consequences of evolutionary pressure promoting overall evolutionary fitness, defined as survival taking precedence over reproduction and direct survival/reproduction taking precedence over long-term survival/growth (12). Because of this precedence being set, injuries or atrophy of tissues such as skin, bone and tendons occur when resources needed for survival are scarce, including situations of starvation (13), and in times of energy depletion due to acute and even chronic inflammation (1). Many CIDs manifest at older age and therefore exert little selection pressure. Our ancestors had much lower life expectancies and rarely suffered from CIDs or the resulting adverse health consequences, and when they did this would hardly have affected survival and reproduction (14). Nevertheless, although inflammation can affect important organs such as the liver and liver inflammatory mechanisms are essential for the maintenance of liver health, it is important to note that even in these situations, hepatic gluconeogenesis is maintained during immune activation, providing the energy required for survival and reproduction (15, 16).

During the preparation of this review, which started in 2008, other authors published the term “selfish immune system” and so we refer to those publications (17, 18). Nevertheless this paper has been written as original and focuses on total different insides of the selfish character of the immune system. The purpose of this review is to give evidence that the immune system overrides the brain in situations of low grade inflammation and that the dominance of the immune system is the major reason for the development of most if nota l
chronic diseases. It is obvious that the comparance between a solid organ such as the brain and a circulatory organ such as the immune system is difficult. Nevertheless, if we do not consider anatomy, both Systems are perfectly comparable at the level of behaviour, health and disease.

1.2. Protective role of the immune system during human evolution

Robust adaption to new environmental challenges involves epigenetic changes that influence rapid (epigenetic; individuals, some generations) and long-term (genetic; generations) adjustment of the phenotype, for instance by epimutation, single nucleotide polymorphisms and gene copy number variation (19, 20). Numerous environmental factors have shaped the human genome, including climate, food and microbial load (21). Although the first two challenges certainly show selective pressure in humans, the main selective pressure seems to derive from pathogens because of their high degree of potential lethality (22).

When hominins began exploring new environments looking for food and scavenging, they were exposed to new pathogens. For example, dead meat, when spoiled, is a perfect source of pathogens such as Escherichia coli, Salmonella and other possible lethal microbes (23). The struggle to survive in new situations led to the development of an incredibly effective and robust immune system. The survival mechanisms evolved at least four times and entailed: upregulation of anti-inflammatory and anti-pathogenic strategies (24), spontaneous physical activity (25, 26), the development of a highly sophisticated behavioural immune system (27), and higher immunological reactivity, when compared with our evolutionary closest counterpart, the chimpanzee (28, 29).

The higher reactivity of the immune system enabled the exploration of new environments and, when necessary, the ability to mount a massive innate immune response to prevent lethal infection (30). This response is extremely costly and would have hardly permitted the further brain growth observed in later hominins if the pro-inflammatory reaction to pathogens had prevailed over the needs of the brain on a chronic basis. The use of the first three strategies might have been necessary because of a much lower energetic cost, thus protecting against pathogenic load, without suffering from the possible secondary damaging effects of a pro-inflammatory strategy (31). It is therefore conceivable that the combination of these three strategies ‘liberated’ energy for larger brains and an expansion of brain functions.

Failure of these three strategies (i.e. upregulation of anti-inflammatory and anti-pathogenic strategies, spontaneous physical activity and the development of a highly sophisticated behavioural immune system) necessitates a protective high-cost pro-inflammatory response.
and the entire body is then at the disposal of the immune system; “prima vivere e dopo filosofare” (first live and then philosophise). This selfish behaviour of the immune system is observed not only in acute inflammation but also in chronic inflammation, the major difference between these two states being a shift from a hypermetabolic state to a hypometabolic state. Figures 1 and 2 show how the immune system puts the body at its disposal in acute inflammatory (Figure 1) and chronic inflammatory (Figure 2) states.

Observing the actual pandemic increase of non-communicable diseases, we consider that it is this selfish behaviour of the immune system that causes the majority, if not all, of these diseases. Although the selfish immune system gave humans the ability to explore the entire world, it now seems to be responsible for most modern diseases, including cardiovascular disorders, autoimmune and neurodegenerative diseases.

**Figure 1. The immune/metabolic response during acute inflammation.**

The increased need for energy during acute inflammation causes a hypermetabolic state and allocation of resources, including proteins and minerals, to the immune system. Insulin levels are down-regulated by inhibition of pancreatic B-cells and glucose can be used by the immune system through development of adaptive insulin resistance of competing organs such as muscles, fat and liver. Short-term use of neurotransmitters by the immune system increases the activity level of the innate immune inflammatory response, ‘helping’ the need to mount an intense but optimal reaction that will resolve in a maximum of 4 to 7 days. Sickness behaviour induced by hyperleptinaemia and pro-inflammatory cytokines further saves energy, induces sleep and adaptive cachexia. The optimal IIS response is short-term and will moderately activate antigen-presenting cells. The adaptive immune system will produce anti-inflammatory memory cells that can be recruited when the host encounters a different immune challenge of the same type. This adaptive immune response generally ends after a maximum of 27 days, leaving sufficient energy to maintain health of the organs disposed of during the start of the immune response. Resolving substances such as protectins and resolvins finish immune activation and homeostasis of the whole body is recovered through normalised energy distribution. Ach, acetylcholine; APC, antigen presenting cell; IIS, innate immune system.
In the modern world, long-term pro-inflammatory activity of the immune system is frequently caused by anthropogenic factors and other conditions that challenge the immune system only weakly, but chronically. In normal situations any inflammation would produce a compensatory immune suppression, which is why low-grade inflammation needs a logical explanation. To maintain pro-inflammatory activity, the immune system itself puts the entire body at its disposal, but at the same time protects the body against multiple organ failure by inducing a hypometabolic state. Cortisol and noradrenaline induce gluconeogenesis and this extra glucose is allocated to the immune system by increasing insulin resistance of competing organs. At the same time, leptin reactivates the immune system, whereas brain regions associated with satiety develop leptin resistance, inducing increased food craving. Low thyroid hormone (rT3>T3) decreases total energy expenditure (protective hypometabolism) and rT3 is needed to fight pathogens. Nerve-driven immunity provides the immune system software (serotonin and dopamine) to maintain pro-inflammatory activity, whereas immune-suppressive mediators are down-regulated (cannabinoids and acetylcholine). Retraction of sympathetic fibres of inflamed/immune tissue and increase of sensory fibres are hardware strategies of nerve-driven immunity. Chronic pro-inflammatory activity is further maintained by a shift from an anti-inflammatory androgenic to a pro-inflammatory oestrogenic state in both males and females. The total picture of this ‘selfish immune system behaviour’ might be considered protective when it does not last too long; it is however highly deleterious when the human body starts developing all kinds of modern disorders such as autoimmune diseases, neurodegeneration and other maladies. Nevertheless, even today it is usually better to develop a CNCD than to die of cancer or infection.
1.3. Acute inflammation resolves itself; controlled resolution

The immune system is self-regulating through negative biofeedback mechanisms, just like any other system in the human body. Acute inflammation induces the production of several substances responsible for finishing the immune reaction, including arachidonic acid derived lipoxins, EPA-derived protectins and DHA-derived resolvins (1), but only if the aforementioned fatty acids, notably the fish-derived fatty acids EPA and DHA, are available in sufficient amounts (32–34). These substances decrease the activity of pro-inflammatory cells of the innate immune system and, at the same time, stimulate migration of phagocytizing macrophages to the danger zone/battlefield (35–37).

Another more intrinsic negative biofeedback signal is lactic acid. The activated immune system uses (cytoplasmic) glycolysis as energy metabolism, in which 90% of glucose molecules are converted into lactic acid, a metabolic shift from mitochondrial oxidative phosphorylation (MOP) to cytoplasmic substrate level phosphorylation (SLP) (38). This metabolic shift seems counterintuitive when considering that only 2 molecules of ATP are generated from 1 molecule of glucose during SLP, whereas MOP yields 36 molecules of ATP. This is, however, conceivable in the light of the velocity of SLP, which is a hundred times faster than MOP (38, 39).

A second benefit of SLP is that the glucose molecule is only partially used for ATP generation. Lactic acid and other macromolecules are metabolites of SLP that confer several favourable positive conditions during acute inflammation that guarantee cell division and cytokine production and render the immune system to a state independent of food and oxygen. The immune system’s capacity to engage in SLP is also known as the ‘Warburg effect’, which not only provides the immune system with fast energy, but also the precursor (glucose) needed for the synthesis of structural elements for the production of all DNA, RNA, organelles and the majority of cell membranes (for an excellent review, see 40). The oxygen-independent fermentation of glucose in the cytoplasm thus leads to the production of amino acids as precursors of proteins, (deoxy)ribose for DNA and RNA, glycerol for lipids and NADPH through the pentose phosphate pathway, needed for the production of phospholipids and glutathione (40). The activated immune system is now capable of growth and proliferation, largely without the need to uptake oxygen and building blocks. Considering the sickness behaviour associated with acute infectious disorders, characterised by cachexia (food absence) and even diaphragmatic breakdown (low oxygen) (41, 42), this makes sense.

Lactic acid supports the role of lipoxins, resolvins and protectins in finishing the inflammatory response in a maximum of 4 to 7 days. Finishing the inflammatory response in time protects the body against possible deleterious secondary effects caused by the immune
system itself. Not only have intrinsic mechanisms emerged to end an acute inflammatory response, but brain-coordinated strategies, including the production of substances such as cortisol (43), certain cannabinoids (44), acetylcholine (45) and catecholamines (46, 47), are able to switch off the immune system. These inhibiting mechanisms are activated through coordinated processes during acute inflammation and the same holds true for the serotonin and dopamine pathways (see chapter ‘behaviour at the disposal of the immune system’). Observing the multiple mechanisms responsible for inhibiting the pro-inflammatory activity of the immune system, it can only be concluded that the inflammatory response should be finalised in time, leading to controlled resolution even after infection (48).

Therefore, why is immune-inhibition absent when people suffer from weak inflammation caused by anthropogenic factors (AF)? AF activate the immune system through indirect pathways and causes a weak, ‘cold’ inflammation, without any of the typical signs of ‘hot’ inflammation (49). Usually adipocytes are activated by AF, such as high caloric food intake and psycho-emotional stress. Although this type of inflammation is not as strong, it still produces a metabolic shift of the immune system, giving rise to the production of substantial amounts of lactic acid, which serves as a potent immune suppressor through the creation of significant acidosis (50, 51). The question therefore remains how the immune system ‘manages’ its activity for years and years, in spite of intrinsic and extrinsic mechanisms that inhibit the immune system and generally protect the body, but especially the selfish brain, against secondary damaging effects.

We suggest that the immune system manages long-term activity by pursuing at least two strategies, firstly to achieve energy (glucose) and secondly to reactivation itself. The first strategy has to induce constant gluconeogenesis and energy allocation to the immune system and the second strategy has to reactivation the immune system. Pro-inflammatory immune system activity depends on several conditions. 1. Pro-inflammatory cytokines have to be produced through activation of the key regulator of the immune system being nuclear factor-κB (NFkB), 2. Immune cell growth and cell proliferation depends on activation of the mammalian target of rhapamacin (mTOR), 3. cytoplasmic glycolysis is needed for the production of macromolecules and energy during immune activation and this requires the activation of hypoxia-induced factor-1 (HIF-1) and 4. The activated immune system demands large amounts of glucose and therefore a higher number of glucose transporters type 1, achieved by upregulation of c-myc (52-54).
Strategies used by the immune system to reactivate itself include:

- Insulin resistance and high insulin levels
- Leptin resistance and hyperleptinaemia
- Low thyroid hormone syndrome
- Catecholamine resistance of the immune system
- Cortisol resistance of the immune system
- Systemic androgens to oestrogens shift
- Peripheral serotonin recruitment
- Peripheral dopamine recruitment

The majority of these different strategies are associated with sickness behaviour exhibited during acute infection (55). This behaviour is characterised by symptoms such as cachexia, fatigue, increased sleep and fever. It suggests that the activated immune system itself is responsible for fasting during infection and 'senses' that food will not be available under these conditions. The outcome is the mentioned shift from MOP to SLP, resulting in immune cell proliferation and activity becoming independent from food intake, all to enable the infection to be cured (56). Chronic activity of the immune system, during which the same metabolic shift occurs, would require a large amount of glucose, which is the basic energy fuel of the activated immune system and, of which, the limited availability constitutes the basic problem in chronic non-communicable diseases (CNCD) (56). This 'nutrition-independent' state may be responsible for the recently evidenced decrease in basal metabolic rate in chronic inflammatory disorders, rendering the subject vulnerable to the development of multiple metabolic disorders, including metabolic syndrome and diabetes mellitus type 2 (57).

In summary, the metabolic shift observed in conditions of an activated immune system renders the system glucose-dependent and will therefore activate all strategies to maintain glucose homeostasis through systemic gluconeogenesis (43). The immune system can apply multiple strategies to maintain activity, although at a low level and puts the whole body at its disposal, including the brain, if necessary. This selfish behaviour has a protective effect during acute infection, but may have a dramatically deleterious effect in the long run, evidenced by the number of people suffering from CNCD in our society (58, 59). Low-grade inflammation should therefore be regarded as the cause for the majority, if not all, cases of CNCD. Treatment should therefore target the strategies used by the immune system to maintain its activity over a prolonged period of time. The only way to provide the correct treatment is to understand the ways the immune system puts the whole body at its disposal, which is the aim of this review.
1.4. The selfish immune system; brain damage leads to more brain damage caused by the immune system and overriding the brain as most dominant organ

The immune system is one of the systems with a high level of biological robustness (60). Some diseases and their effects produced by the robust character of the immune system will not necessarily benefit the host, but that is the price to pay. We provide evidence that the mechanisms leading to pathologies affecting the whole body, including the brain, should be considered robust and part of the survival strategies developed during hominin evolution (61).

The interactive neuro-endocrine-immune system evolved to cope with acute immunological challenges such as infection and wound healing, but is also activated by non-immunological danger for which it was not designed (2, 62). Theories explaining biological priorities consistently put the human brain in first place (63, 64). However, brain functions, blood circulation and anatomy are disrupted in those suffering from Alzheimer’s disease, Parkinson’s disease, fibromyalgia syndrome (FMS), chronic fatigue syndrome (CFS) and depression (65-68), which suggests that certain pathophysiological processes override the protective behaviour of the selfish brain.

FMS depression and Alzheimer’s disease (AD) are obviously not diseases of choice, but may rather reflect the involuntary alternative of suffering from a low energetic state leading to a non-permissive brain disorder (e.g. AD, depression and FMS), rather than dying from multiple organ failure (69-71) caused by a chronic activated pro-inflammatory immune system.

Rogers (72) stated that “Inflammation seems useful when controlled, but deadly when it is not. For example, ‘head trauma may kill hundreds of thousands of neurons, but the secondary inflammatory response to head trauma may kill millions of neurons or the patient’ and the same holds true for people who suffer a stroke (73, 74). The inflammatory response of the immune system causing severe secondary damage to the brain after traumatic brain injury or ischaemic stroke seems maladaptive in the face of the ‘selfish’ brain hypothesis. It would, however, correspond with the ‘selfish’ immune system hypothesis, which states that danger gives precedence to the immune system, and thereby overriding the selfish brain.

It may even be the case that dramatic inflammatory response following brain injury is not caused by the injury itself, but by the presence of infectious pathogens. Pre-existing infection is present in one third of clinical ischaemic stroke patients (75). Clinical data suggest that stroke risk peaks at three days after infection onset and that this risk remains high for three months (76). Almost three out of every four people in the world who suffer a fatal stroke live in developing countries and malaria, Chagas disease and Gnathostomiasis seem to be the major
causes for this surprising fact (77). Children and young adults are less susceptible to all-cause strokes with the exception of infectious stroke and diseases such as sickle cell disease. A recent case-control study revealed that pre-existing infection is an independent risk factor for stroke in 33% of affected children also in developed countries, such as the USA where 2,400 children suffer from strokes every year (78). These data suggest that a possible pathogenic presence ‘programmed’ a severe pro-inflammatory immune response when the brain or the heart muscle are damaged. The selfish behaviour of the immune in these cases should be considered as adaptive but also possibly deleterious. Nevertheless the fact that people suffering from immune suppression after stroke are highly susceptible for sepsis and death (79) suggests that the selfish behaviour of the immune system should be considered as needed when damage to the heart or the brain has been done.
2. It’s all about energy – The selfish immune system

2.1. Robustness and energy reallocation between visceral organs, muscles, the immune system and the brain

The immune system is one of the energetically most costly systems in humans when ‘activated’. Consequently, immune metabolism has a profound effect on the functioning of the body. Metabolic conflicts between organs seem to explain the emergence of several disorders and, more specifically, modern non-communicable diseases such as autoimmune disorders (80).

Anthropogenic danger signals, such as psychoemotional stress and sleep deprivation, are capable of activating the immune system and a chronically activated immune system demands high energy, protein and immune-specific minerals such as calcium (2). Such high costs would never have permitted the development of the phylogenetic newer metabolic expensive brain in general and, more specifically, the neocortex during evolution. A recent review describes how exercise (searching for food, water and shelter) in primates and early hominids produced a shift from a pro-inflammatory immune reaction with a high metabolic demand to an anti-inflammatory response with a low metabolic demand (31). This shift made it possible to allocate energy to other organs e.g. the brain without large amounts of energy, proteins and minerals having to be invested in the immune system, whilst at the same time maintaining protection against microbes.

2.2. Energy and energetic conflict as the driving force behind evolution

Changes in energy allocation between organs are a consequence of energy conflicts that affect all animals, but non-human primates and humans in particular (81, 82). Several scenarios have been proposed, with the brain being the organ to benefit from loss of colon length and high caloric dense food [the expensive tissue hypothesis] (81, 82)], a decrease in muscle mass, an increase in fat mass (49), cooking (83), human locomotion costing less energy (84) and, very recently, a change in the expression of glucose transporters beneficial to the brain (85). To our knowledge, as yet it has not been suggested that immune function may also have benefitted from a smaller gut, a lower energy demand for locomotion, an increase in fat mass and tissue-specific differences in the expression of glucose transporters. The latter entails a higher expression of GLUT1 in activated immune cells, when energy is needed to protect against pathogens and other immune challenges (86), at the expense of GLUT4 expression in muscles and adipocytes (87-90).
The work of Fedrigo et al. (85) shows that glucose transport capacity has been essential for brain growth and function during human evolution. They demonstrated that human brain cells express more activity of the SLC2A1 gene, which is the genetic code for the production of GLUT-1 glucose transporters compared with the chimpanzee and macaque (human > chimpanzee > macaque). At the same time, SLC2A4 expression in muscle is significantly higher in chimpanzee > human > macaque.

Logical reasoning makes it plausible that a concomitant per gram tissue reduction of SLC2A4 in skeletal muscle and an increase of SLC2A1 in the brain will lead to higher glucose uptake by the brain at a given plasma glucose concentration. This would result in a shift of glucose allocation away from the body (strong) and towards the brain (smart).

2.3. Glucose to the immune system – prioritising energy guidance

Along a similar vein, the same holds true for glucose allocation to the immune system. The energy demand of lymphocytes and leukocytes increases dramatically upon activation (2, 4) and all activated immune cells express GLUT1 glucose transporters (86, 91). Higher expression of GLUT1 will promote energy allocation to the immune system, which could be considered to be an ‘energy demand reaction’ (92). Glucose allocation to the immune system maintains its function even under strong energy restriction (80).

The foregoing demonstrates that activation of the immune system through danger signs will attract and redistribute energy, favouring the brain and the immune system. Prolonged activation of the immune system (as has been observed in people with CID.s) would allocate glucose chronically to the immune system through immune-controlled down-regulation of GLUT1 transporters at the level of the blood-brain barrier and would decrease GLUT4 transporters at the level of muscle and adipose tissue (93-96).
3. **Evolution shaped the selfish immune system**

3.1. **Evolution and the human selfish immune system - over-reactivity of the human immune system when compared with chimpanzees**

It has been shown that the human immune system is relatively over-reactive when compared with our closest evolutionary relative, the chimpanzee (97). The increased activity of the human immune system holds true for both the innate and the adaptive immune systems (98). It seems that all major cell groups of the human immune system show lower levels of mediators capable of down-regulation of the immune response against pathogens and phytohaemagglutinin (28). These mediators, called inhibitory sialic acid-recognising Ig-superfamily lectins (SIGLEC), are expressed on most immune cells including B lymphocytes (99). The difference between chimpanzees and humans is three-fold. Firstly, humans express different SIGLECs; secondly, humans exhibit lower SIGLEC numbers and little or none are present on T lymphocytes, and thirdly, they show a lower production rate of inhibitory SIGLECs when challenged with pathogens or other immune stimuli (99, 100).

The observed development of a more reactive immune response in humans is probably a consequence of being faced by unique immune challenges to numerous pathogens through scavenging, increased population density, hunting and migration (101, 102).

3.2. **The selfish brain is less selfish than the much more ancient immune system**

The brain is selfish in almost every situation, including mild and severe stress (103) and multiple studies support the ‘selfish brain’ hypothesis. Acute mild mental stress requires 12% additional energy from the human brain (104) and the same holds when humans are challenged by intense exercise (105). The group of Peters showed that social stress also augments the brain’s energy need (106) and that the brain switches to the use of ketone bodies (107) and lactic acid (107) when glucose is unavailable. Therefore, several lines of research give evidence for the ‘selfish brain’ hypothesis in which it is stated that brain energy is maintained through multiple pathways, including activation of central stress axes and the use of multiple energy sources (glucose, ketone bodies, lactic acid). Immune activation also produces activity of central stress axes and a state of high arousal of the central nervous system, the purpose being to sense and avoid further danger (108). An acute inflammatory response produces energy allocation to the immune system until this is resolved, and only when the system is challenged by mono-metabolic danger signals (108). However, multi-metabolic risk factors produce an energetic conflict between the immune system and the brain. The combined need for resources (energy-producing macronutrients, blood, oxygen)
of the stressed brain, of the activated immune system and of other organs responsible for maintaining organ functions during multiple metabolic signalling challenges, caused by psychogenic, psychosocial and physical factors at the same time, would probably override the maximum capacity of energy uptake by the gut and, although speculative, would demand a maintained heart rate of around 180/minute and a chronically increased blood pressure at around 160/120 mm Hg. This would lead to severe damage to the heart and probably the brain, which is contrary to the evolutionary drive of maintaining brain function and anatomy against at any cost (106). The only feasible response to maintain life throughout chronic situations of high energy demands of ‘conflicting’ organs, is the ‘creation’ of an organ-specific low thyroid hormone syndrome and other adaptations with the purpose of lowering the activity of all organs and puts the body at the disposal of the immune system. This is evidenced by the development of immunological sickness behaviour, immunologically induced secondary damage of vital organs, protective depression, gluconeogenesis by the liver and kidneys, and the use of metabolic hormones and neurotransmitters by the immune system to benefit its pro-inflammatory activity and thereby protect against possible lethal pathogens (109, 110, 111, 112).

It is definitely true that the brain ‘behaves’ selfishly in almost every situation, including an energy deficient intra-uterine environment. Nevertheless, although it seems that one of the most fundamental biological drivers in humans is supplying the brain with nutrients and energy, how is it possible for people to suffer from diseases related with lack of brain energy? Something has to be so wrong that it may even cause a reaction that overrides this interest and ‘accept’ the collateral damage to the selfish brain. Acute sepsis, severe burn wounds, multiple traumata and major surgery are known to allocate up to 100% of resting energy expenditure to the immune system, but these are acute situations which often lead to instant death, even in children (3, 4, 113-116). Neurodegenerative disease, fibromyalgia, and chronic fatigue disorders develop slowly and should therefore be caused by factors that demand long-term energy allocation to systems other than the brain, e.g. the immune system, ultimately affecting the transport of resources to the selfish brain. Sedentary lifestyle, overeating, childhood abuse, oral sepsis, chronic life stress, leaky barriers, perceived social stress, environmental toxins, social jetlag, meal frequency and even father-daughter conflict all activate the immune system and an energy demand response based on activation of the SAM and HPA axes (117-121). This latter response should provide energy for, primarily, the brain and secondarily, the immune system. When brain allocation fails, brain functions and possibly anatomy will be disturbed. The latter occurs in people suffering from acute infection, but also in those suffering from Alzheimer’s disease, FMS, CFS, depression and other diseases affecting the central nervous system. It seems that multiple metabolic danger signals produce a state mimicking acute life threatening danger, allocating energy to the immune system and disposing of energy from the rest of the body, including the brain.
4. Genetic and environmental evidence supporting the hypothesis

4.1. Genetic evidence for the selfish immune system hypothesis

Depression and other maladies, including FMS and neurodegenerative disorders, such as Parkinson’s and Alzheimer’s diseases, are related to increased immune activity (122, 123, 124). All of these disorders seem to have a genetic predisposition and the majority of the genes related with neuro-degenerative disorders and depression influence the immune system. Raison hypothesised that if depression is related to certain polymorphisms, then these genes are primarily protective in the face of infection (for review see 125). The same holds for genes related to Alzheimer’s disease and FMS.

The gene most widely accepted to be associated with Alzheimer’s disease susceptibility is ApoE 4 (apolipoprotein E4), although many others have been proposed as Alzheimer genes (126). Classical functions of apolipoprotein E relate to metabolism (transporter of lipids in the periphery and in the central nervous system (127)). More recently, it was found that ApoE influences the innate and adaptive immune systems (128). The overall influence of ApoE on the innate immune system is complex and depends on ApoE polymorphism. ApoE4 induces an inflammatory response, whereas ApoE3 inhibits inflammation and enhances repair (see references in 128). The pro-inflammatory activity of ApoE4 is classically seen as deleterious, but can also be considered protective in the light of the pathogen-host defence (PATHOS-D) hypothesis proposed by Raison and Miller (125). In our recent evolution, the share of the ApoE3 allele appears to have increased at the expense of the ancestral ApoE4 (129). ApoE3 has spread significantly in first world populations, whereas the prevalence of ApoE4 is still very high in cultures historically exposed to disease-causing pathogens (116, 130-132). The observation seems consistent with the protective role of ApoE4 against infection. The Yoruban population in Nigeria has a 70% lower incidence of dementia when compared to African Americans. This difference is probably caused by the lifelong low-fat diet of the Yorubans (133).

Candidate genes possibly associated with increased susceptibility to fibromyalgia syndrome have been reported, but no definite conclusions can be drawn as yet (134). The serotonin transporter gene SLC6A4 is perhaps the strongest candidate in terms of its relation to FMS (51, 135, 136). Two major SLC6A4 polymorphisms have been identified. The short allele carrier produces a protein, which is less efficient in the reuptake of serotonin and carriers show an increased risk for the development of depression when facing psychosocial challenges. Carriers of the short allele further show higher levels of circulating pro-inflammatory cytokines compared with anti-inflammatory cytokines (IL-6/IL-10 rate) when challenged
with a psychosocial stressor (137). Another disease with an immunological genetic background is celiac disease. Celiac disease is caused by the intake of gluten and it’s interaction with a great number of genes, of which most are related with an increased reactivity of the immune system (138). More specific, it are alleles related with IL18, IL23, IL2 and IL12 that increase the susceptibility for celiac disease, but at the same time people expressing these genes are probably better protected against pathogens, including virus and bacteria (139)

4.2. Pathogens shaped genetics to the benefit of the immune system and pathogen load prioritises the immune system over the brain

If the PATHOS-D hypothesis is correct, that the microbial world is co-responsible for the chronically increased activity of the immune system and disposal of expensive brain functions, then pathogenic load should not only affect brain function and anatomy of older people, but also younger individuals. Evidence for this supporting the PATHOS-D hypothesis comes from studies investigating the development of intelligence during human evolution in general and, more recently, the past two hundred years. A recent study of Eppig (140) showed that infectious disease and the consequent immune activity is the most important predictor of lower intelligence in almost every population on earth. Nutrition was also correlated with IQ, but became insignificant when corrected for infection. The connection between pathogen load, infection and intelligence seems plausible considering the high energetic cost of infectious disease. (141).
5. **Long-term immune activity: the need for reactivation and fuelling strategies**

5.1. **Evolutionary stored energy limits the timescale of immune activity**

Both the innate and adaptive immune responses are normally self-limiting (142). The self-limiting timeframe of 28 days is probably based on the energy-resource model. A (much) longer massive activity of both systems would lead to severe secondary damage and even death because of sustained energy deficit of vital organs including the liver, kidneys and heart muscle (143). Recruitment of the adaptive immune system, although very expensive at first exposure time (141), and the generation of immune memory, should be considered beneficial from an evolutionary point of view, because of the shortening of the immune response and protection against energy depletion, when the host is newly exposed to the pathogen (64). Chronic low-grade inflammation literally means long-term low activity of the innate immune system and lack of the production of self-limiting substances, such as resolvins and protectins (142). Chronic low-grade inflammation probably starts after a non-optimal acute inflammatory response (supranormal or subnormal) and when self-limiting mechanisms or strategies fail (143). Sterile wounds, low pathogenic load and non-specific immunological challenges such as psychogenic stress activate the immune system, but lack the strength of optimal immune activation which would normally lead to complete resolution (144). Nevertheless if the factors that activate the immune system in a subnormal manner are not resolved, the capacity to maintain immune activity is essential for survival, as evidenced in people suffering from inflammation-associated immune suppression. People suffering from inflammation-associated immune suppression (IAIS) are highly susceptible for the development of different types of cancer and secondary infections, and IAIS significantly increases the mortality rate (145, 146). IAIS is induced by immunological intrinsic (e.g. lactic acid), but also multiple brain derived strategies including the activation of the parasympathetic nervous system. This is where the immune system has to put the body at its disposal, thereby overriding the selfish brain.

5.2. **The consequence: the whole body at the disposal of the selfish immune system**

The way in which the immune system puts the body at its disposal might follow a coordinated sequence. The initial activation of energy demanding central stress axes allocates resources to the immune system through induction of gluconeogenesis and insulin resistance of competing organs, such as, bone, muscle, adipose tissue and the liver (1, 40). Once the innate immune system (maximum 4-7 days) and, when necessary, the adaptive immune system (27–42 days) has/have triggered the immune response, problems should
have been resolved. If immune activation is required for a longer period of time, this induces further insulin resistance/hyperinsulinemia, hyperleptinaemia/leptin resistance, and hypercortisolism/glucocorticoid resistance. The possible hypermetabolic state produced by the constantly activated immune system could cause multiple organ disorders, failure and even death (MODFD). The development of a low thyroid hormone state (rT3>T3) protects the body against MODFD, putting homeostatic regulation at the disposal of the immune system. The pro-inflammatory activity can also be maintained by higher aromatase activity and the production of pro-inflammatory oestrogens (see below): a further step in putting the whole body, including the reproductive system, at the disposal of the immune system.
6. **Fuelling and reactivation strategies of the immune system**

6.1. **Thyroid hormone prevents multiple organ failure during hypermetabolism and maintains immune homeostasis; thyroid hormone at the disposal of the selfish immune system**

Immune activity depends on aerobic glycolysis (147). It is only possible to maintain aerobic glycolysis when the intrinsic inhibitory pathways of the immune system can be overruled. Low T3 and high rT3 can maintain aerobic glycolysis in immune cells. Thyroid hormone T3 induces mitochondrial activity in all kinds of cells, including immune cells (148). T3 can even strongly activate mitochondrial oxidation in cancer cells and render them more sensitive for chemotherapy (149). Intracellular T3 would therefore inhibit the inflammatory activity of the immune system, which would be highly deleterious during severe infection or other immunological challenges. Extracellular T4 and T3 are necessary for immune activation (150), but intracellular T4 is converted by deiodinase 3 (D3) into rT3 and T3 is rapidly downregulated by the same enzyme, preventing mitochondrial activation and maintenance of cytoplasmic substrate level phosphorylation through upregulation of D3 by the pro-inflammatory cytokine IL-6 (151). The final state is that of a low thyroid hormone syndrome (LTHS).

LTHS does not only benefit the immune system, but is also protective against the possible secondary damage of chronic immune system activation. These rather deleterious effects of immune activation on other organs and tissues is prevented by down-regulation of the conversion of T4 into T3 both systemically and tissue specifically, causing the reduction of overall metabolic rate, but especially the activity of organs less important for direct survival during immune activity, such as muscle tissue, liver, kidneys, the heart muscle and the digestive system (152-154). The lower activity of these organs protects them against acute organ failure and sudden death of the host. The protection and lower metabolic rate is a product of low thyroid syndrome and high reverse T3 (rT3) (151). This state is protective initially, but can be deleterious in the long run (155).

A recent discovery by the group of Klein and Schaefer has shed new light on the interaction between the immune system and metabolism. Their group proposed a new model of controlled metabolic regulation by an activated immune system (156-158). They showed that TSH can be produced by several tissues other than the thyroid gland. Dendritic cells (DCs) and several cells from the small intestine are capable of producing TSH and this happens mostly during bacterial or viral infection (159). Certain leukocytes also produce TSH, but with a slightly different structure and this hormone has been named TSHbeta splice variant (160). The TSHbeta splice variant seems to change the thyroid phenotype inducing a shift from T3 to rT3. This shift decreases total body metabolism because of lower systemic T3 (159) and
Initially saves the brain by continued conversion of T4 to T3 in the brain itself (161).

The possible function of D3 expression in activated innate immune cells is intriguing. Thyroid hormones (TH) play a role in differentiation and proliferation of cells, with high T3 inducing cell differentiation and low T3 inducing cell proliferation. Granulocytes are short-lived, fully differentiated cells that migrate to the site of infection and do not proliferate, which may argue against a role for D3 induction in differentiation or proliferation of activated granulocytes. Studies in the 1960s suggested a role for thyroid hormone in the bacterial killing capacity of leukocytes. Iodide in combination with hydrogen peroxide (H2O2) provides one of the most effective antibacterial substances of the immune system. Thyroid hormones are an important source of iodide, and leukocytes generate inorganic iodide by the uptake of iodide and by de-iodinating T4, (162). In combination with the recent demonstration of D3 induction in infiltrating leukocytes during infection, we suggest that D3 induction helps to generate iodide as part of the innate immune response (150). Studies in S. pneumonia-infected D3 knockout mice indeed showed a defective bacterial clearance compared with wild-type mice, which supports this hypothesis (163). Further evidence is given by the work of Kwakkel et al, showing a dramatic increase of D3 production by neutrophils when challenged with bacterial LPS (164).

The resulting state of immune activation, low T3, high rT3, combined with the energy demand reaction of the HPA axis and the sympathetic nervous system maintains immunological homeostasis during prolonged stress. The brain will maintain anatomy and function as long as brain metabolism can be guaranteed. The same holds for the immune system, although long-term stress and inflammation suppress immune activity (46). The latter situation could expose the host to possible infection and death. Protection of the host integrity will now depend on the use of alternative mechanisms to postpone this dangerous state whilst maintaining pro-inflammatory immune activity. This would be the time at which the immune system 1. puts every possible organ at its disposal to guarantee its own metabolic homeostasis, 2. induces resistance to hormones with pleiotropic immunological functions (leptin, insulin, cortisol) and 3. produces a state of nerve-driven immunity, putting almost all neurotransmitters, including dopamine, serotonin, acetylcholine, glutamate and GABA at its disposal (165-169).

6.2. **Gluconeogenesis and glucocorticoid resistance at the disposal of the immune system**

Endogenous cortisol has several effects on the immune system, including suppression through activation of the inhibiting factor kappa B (IkB) (170), apoptosis of immune cells that are no longer needed (171) and migration of immune cells to the so-called ‘battlefield’
(dangerous zone) or back into the ‘barracks’ (lymph knots, bone marrow, thymus) (172) and through activation of macrophage migration activating factor (173). Intact cortisol signalling in the immune system would lead to suppression of the immune system, which is why immune cells show an intrinsic mechanism to develop cortisol resistance, which is essential during acute infection but at the same time co-responsible for low-grade inflammation (174).

Glucocorticoid resistance (GR) of the immune system leads to hypercortisolaemia and constant gluconeogenesis. The glucose produced by GR-gluconeogenesis can cover the energetic needs of the selfish immune system. GR-gluconeogenesis can be induced in muscle, the liver, kidneys and perhaps even the pancreas (175-178). So GR serves two basic strategies to maintain immune activity: immunological GR prevents inhibition of the immune system and GR-induced hypercortisolaemia increases glucose production, necessary for the constant nourishment of chronic active selfish immune cells.

Glucocorticoid resistance itself seems to protect the host against possible viral infection, including HIV, by maintaining high activity of the anti-viral Th1 component of the adapted immune system (179, 180), although GR can be highly deleterious (181). The GR observed during chronic inflammation is universal and mostly occurs along with another ancient protective mechanism: insulin resistance (182). Cells of the immune system show inherent genetically imprinted resistance mechanisms, which protect the body against the immune-suppressive effects of glucocorticoids, although side-effects can be severe, including chronic leukaemia (174).

GR is observed in rheumatoid arthritis, inflammatory bowel disease and COPD and is mostly considered deleterious (183). Treatment of these diseases normally focuses on increasing cortisol sensitivity (184) with contrasting results (1). Increasing GC-sensitivity can even lead to higher mortality when animals are challenged with pathogens such as E. Coli (185). If intrinsic or acquired GR conveys protection against pathogenic load, than asthma, rheumatoid arthritis and inflammatory bowel disease should be associated with increased pathogenic microbial load. Indeed, the group of Siala showed that reactive and undifferentiated oligoarthritis is associated with the presence of a high number of bacteria in the synovial fluid (186, 187). Patients with arthritis also show a high incidence of glucocorticoid resistance (188). Those with chronic asthma present higher bacterial colonisation of the lower airways, linked to the severity and duration of asthma (189), whilst GR is also a characteristic of asthmatic patients (190). Inflammatory bowel disease (IBD) normally evolves with pathogenic bacteria (20) and, as mentioned above, patients suffering from IBD also show a high prevalence of GR (191).
It therefore appears that GR prevents suppression of the innate immune system and the glucocorticoids-induced shift from Th1 to Th2 activity of the adapted immune system (192), thus maintaining protection against microbial infiltration and infection. GR serves the selfish immune system to maintain activity, nourish itself with glucose, but with just one purpose, which is to protect the individual from lethal infection.

6.3. Leptin and insulin at the disposal of the selfish immune system

Leptin and insulin are needed to maintain long-term activity of the immune system and the immune system itself increases the production of leptin by adipocytes via TNFα signalling (193). Leptin is highly inflammogenic (194) and hyperleptinaemia, together with central leptin resistance, maintains pro-inflammatory activity and energy allocation to the immune system (195, 196). Proinflammatory cytokines induce leptin production by adipocytes, as does food intake.

Adipose tissue is present in immune-cell-harbouring tissues, such as lymphoid organs, bone marrow and adipocytes that infiltrate wounds (34) and so adipocyte derived leptin can have direct influence on immune cell functioning.

Leptin activates all types of immune cells and increases glucose uptake during immunological activity. The principal target of leptin-induced immune cell reactivation is the key immune response regulator nuclear factor-κB, responsible for transcription of genes encoding for IL1, IL6 and TNFα (197). In summary, leptin activates the immune system through different pathways with a focus on the innate immune system and Th1. Under physiological circumstances, this leads to increased protection against infection and pathogenic growth. During low-grade inflammation, leptin should be considered to be a re-activator, which can perpetuate immune activity.

The strategies used by the immune system to maintain its activity and guarantee glucose availability could merely have evolved for their beneficial effects; a basic rule in evolutionary biology. This also holds true for the leptin and insulin responses observed during acute and chronic inflammation. The leptin response during inflammation supports different protective traits. Hyperleptinaemia during immune activity informs the brain about the adequacy of long-term energy stores in adipose tissue, asking for/demanding permission to produce a costly fever reaction and a short-term hypermetabolic state, following immune activation (198, 91). The hyper-leptinaemic state will also produce inflammatory cachexic behaviour, which is protective at the start when facing an acute inflammatory response, but could be deleterious when chronic, as is observed in patients and animals with chronic kidney inflammation (199).
Long-term hyperleptinaemia leads to central leptin resistance. Several researchers noted that hyperleptinaemia is required for the development of leptin resistance (200). Central leptin resistance is responsible for an increased risk of overeating (201) and overeating rapidly produces leptin resistance (202). Central leptin resistance can be considered to be an evolutionary advantage when energy availability is low, or when the need for energy is chronically increased as observed during prolonged immune activity (203). The beneficial effect of hyperleptinaemia and LR is observed in different situations. Hyperleptinaemia and LR protect against cardiovascular disorders by preventing lipid deposition in the heart muscle itself (204, 205), although recent publications have challenged this view (206). The influence of leptin on the anti-pathogenic function of the immune system has recently been demonstrated in two new studies from the same group (207, 208). Children with low leptin levels are more susceptible to infection (209). The required pro-inflammatory effect of leptin to fight against pathogens has also been demonstrated in a recent in vitro study (210). The overall effect of leptin on the immune system seems to be permissive, which implies that intact leptin-signalling towards the immune system maintains Th1-Th2 functioning and, if necessary, 'permits' pro-inflammatory activity (211).

Like leptin, insulin is also recognised as a pleiotropic hormone. Energy demands of the brain, or the immune system during starvation, infection or stress are covered by gluconeogenesis and the temporary development of insulin resistance of various organs, caused by proinflammatory cytokines and stress hormones (212, 213). Energy allocation to the immune system is achieved by activating the energy-demand stress systems (sympatho-adrenomedullary, axis, SAM and hypothalamic-pituitary-adrenocortical axis, HPA) and stress systems-induced gluconeogenesis. Hyperinsulinaemia precedes stress-induced and inflammation-induced insulin resistance (214). Hyperinsulinaemia is seen immediately after a stress challenge and/or direct immunological activators, such as injuries and pathogen invasion (215). Low insulin levels increase the susceptibility to develop infections, suggesting that insulin protects against pathogens (216). Acute inflammation produces down-regulation of insulin levels through inhibition of pancreatic β-cells (217) and also insulin resistance in competing organs for glucose uptake (such as liver, muscles and adipose tissue (212). In this way, glucose becomes available for the energy-demanding immune system. Chronic inflammation maintains the state of insulin resistance and enhances insulin production, leading to hyperinsulinemia (218). In the latter situation, glucose remains available for the immune system and insulin can now be used as reactivator through the mTOR pathway in immune cells, protecting against possible infections which is, however, deleterious in the long run (219). Insulin can also upregulate the specific glucose transporters on immune cells, including GLUT1, GLUT3 and GLUT4, thereby increasing glucose uptake by leukocytes and lymphocytes (220). Insulin signalling through insulin receptors on immune cells stimulate the IRS-1/PI3K/AKT pathway that activates mTOR1 and mTOR2 (221). mTOR signalling
recruits c-myc, NFkB and HIF1, facilitating further glucose uptake, production of pro-inflammatory cytokines and maintenance of cytoplasmic aerobic glycolysis, respectively (222-224).

It seems clear that leptin and insulin pathways are capable of fuelling and reactivating the immune system, not only during acute infection, but also to maintain long-term activation. The capacity of redistributing glucose to the immune system and away from peripheral tissues mediates the immune response and has been crucial to human survival. In other words: leptin and insulin signalling beneficial to immune system activity are vital for survival, and are meanwhile also responsible for chronic low-grade inflammation and its associated diseases.

6.4. The reproductive system at the disposal of the selfish immune system

The combined metabolic shift produced at the disposal of the pro-inflammatory activity of the immune system is directly responsible for the pro-inflammatory systemic hypoandrogenic state observed in individuals suffering from low-grade inflammatory disorders (225). This systemic hypoandrogenic state is not produced because of lower testosterone production in the sex organs. To the contrary, obese males, characterised by increased plasma leptin and low-grade inflammation, exhibit a higher testosterone-dependent risk of prostate cancer (226), while the same holds true for the polycystic ovary syndrome in females (227).

The testosterone boost produced by metabolic hormones precedes the increased systemic and tissue-specific conversion of testosterone into pro-inflammatory oestrogens. This shift has been observed in different diseases, including obesity and inflammation-related breast cancer (228).

The systemic shift from testosterone to oestrogens could benefit the anti-pathogenic pro-inflammatory activity of the immune system. Males with higher testosterone levels are more susceptible to parasite infection, microbial transmission (229) and have decreased resistance against tick infection (230). Conversely, low testosterone protects against bacterial infection in general and specifically against prostate infection (231). It is therefore conceivable, but at the same time striking, that both males and females exhibit higher aromatase activities during inflammation as evidenced in patients with rheumatic diseases, characterised by high pro-inflammatory oestrogens and low testosterone levels (232). The protective effect of oestrogens against microbial infiltration and infection is supported by epidemiological data showing that postmenopausal women are disproportionately susceptible to recurrent urinary tract infections (233). Urinary tract infections (UTI) in elderly patients are more treatment resistant
and oestrogen replacement diminishes UTI frequency (234). Chronic inflammation and stress lead to low testosterone and high oestrogens in men (235) and high oestrogen levels protect against possible infection.

A possible negative effect of this shift from testosterone to oestrogen is the loss of fertility (41). Obese men, characterised by high aromatase activity in adipocytes (236) and low-grade inflammation (237), have lower fertility (238). Individuals engaged in a chronic struggle against pathogens rather not reproduce, preventing damage to offspring, which is beneficial to overall reproductive success (125). The septic danger posed by non-sterile wounds is included in the selective pressure factors shaping human behaviour (phenotype) and genome (genotype). It has been shown that the shift from testosterone to oestrogen in the skin is highly protective against pathogenic infection, prolonged infection, wound healing and overall cutaneous repair (239). It is consequently conceivable that the immune system also puts the reproductive system at its disposal. Immediate survival overrules reproduction. Once again, the immune system dominates the whole body, including the timing of reproduction and, if necessary, protecting genetically-related individuals against possible pathogenic damage, or damage to the immune system itself (240). Oestrogens activate the immune system through several mechanisms including stimulation of NFkB, c-myc and mTOR, facilitating immune cell proliferation and inflammatory activity (241).

6.5. Behaviour at the disposal of the immune system: serotonin-dependent reactivation of the immune system

The observed changes in behaviour during inflammation suggest that the immune system actively affects neurophysiological function, putting behaviour at its own disposal. Pro-inflammatory cytokines such as TNFα, IL-1 beta, and IL-6 produce adaptive behavioural effects when entering the brain (55). Sickness behaviour includes social withdrawal, increased sleeping time, fatigue and exercise avoidance. This reduces energy uptake by muscles and the brain and this is reallocated to the immune response.

Several pathways explaining inflammation-induced sickness behaviour have been proposed and all of them probably contribute to this state (120). Sickness behaviour not only benefits the host’s immune system in terms of energy/resource reallocation, but also helps the immune system to fight pathogens and restore homeostasis when immune activity is no longer needed (55). Pro-inflammatory cytokines (IL1β, IL6, TNF-α and IFNy) and stress hormones, produced during inflammation, activate tryptophan 2,3-dioxygenase (TDO) and indoleamine 2,3-dioxygenase (IDO), affecting serotonin production from its precursor tryptophan and favouring the production of kynurenine and quinolinic acid (242). The resulting serotonin depletion is considered to be one of the factors causing sickness.
behaviour (243), which, when considered from a proximate prospective, could be considered a maladaptive response. The latter is supported by showing that quinolinic acid, produced by cells in the central nervous system, is highly neurotoxic and associated with the development of numerous neurodegenerative conditions, including Parkinson’s and Alzheimer’s diseases (215).

An evolutionary explanation for the underlying mechanism considers that upregulation of IDO and TDO during acute inflammation protects the host significantly by depleting tryptophan and efficiently suppressing the growth of pathogens and malignant cells (120). Serotonin further inhibits activation of the sympathetic nervous system (244), while SNS is needed for energy production and its allocation to the brain and the immune system during inflammation. Inhibition of serotonin production during inflammation will therefore favour SNS activity and energy production/allocation to the immune system.

Serotonin is present in high concentrations at the sites of inflammation and is used by activated immune cells as co-stimulator through reuptake via the serotonin transporter protein (245). This is beneficial, considering the need to mount an optimal immune response during inflammation, but could be deleterious in the long run and cause several disorders including autoimmune diseases (245). IDO will not only deplete tryptophan but also serotonin and both pathways will inhibit the immune system activity when it is no longer needed, thereby recovering tissue homeostasis and facilitating tissue repair. A feeling of sickness and even pain are common consequences of this highly effective neuroimmunological reaction but that is the price to be paid (246).

The total picture of inflammation-caused sickness behaviour should be considered beneficial to the host. Only when inflammation is supramaximal, such as in sepsis, or when inflammation lasts too long, sickness behaviour has more of a negative impact because of the possible damage caused by immune system dependent pathways. These deleterious effects to the brain show that, if necessary, the immune system will take over and override the interests of the selfish brain, supporting the ‘selfish immune system’ hypothesis.

6.6. Behaviour and immune system co-evolution: dopamine-dependent reactivation of the immune system

The use of dopamine as an immunological co-stimulator has been studied extensively and is of high clinical importance (247). Dopamine recruitment by the immune system has profound effects on inflammatory behaviour. Humans have engaged in exploring new environments and this demands several traits, including curiosity (25), a large brain and immune protection.
Dopamine is considered to be the main neurotransmitter responsible for curiosity (248), novelty seeking (249), motivation and aggressiveness (250). A polymorphism of the dopamine receptor D4 (DRD4) is associated with novelty seeking, risk-taking and increased exploratory behaviour (251). Novel environments produce new immunological challenges, including climate, food availability and pathogens (252). The long allele of the DRD4 receptor is related to the migratory distance from Africa. Matthews and Butler (251) suggested that this allele has been positively selected, as opposed to genetic drift.

Other behavioural traits, in addition to the association of 7R DRD4 polymorphism with environmental exploration and novelty seeking, are increased anger and a decreased feeling of disgust (253). Disgust is amongst the most intensively investigated emotions belonging to the behavioural immune system (248). Immune defence is usually a reaction following tissue damage or some pathogenic infection. It is highly costly and intense and long-term activity could result in secondary lesions and even multiple organ failure. Humans have explored new environments with constant new immunological challenges throughout evolution. The development of a pro-reactive behavioural immune system, preventing contact with possible pathogens could have been beneficial to save energy and guide them to important other physiological functions, including those of the brain and skeletal muscles (254, 27). Disgust as a proactive strategy to avoid disease, produces aversion to a wide range of factors. High levels of disgust, i.e. increased activity of the behavioural immune system, produce neophobia (255), rejection of other individuals, decrease in mating behaviour (256), food neophobia (257), prejudicial attitudes to old people (258) and even discrimination (259).

The behavioural immune system (BIS) can be very sensitive and dominate free will. However, individuals, carrying the longer allele of the DRD4 gene exhibit a higher level of novelty seeking, less disgust and more spontaneous activity (236). This implies that people with increased exploratory behaviour through DRD4 polymorphism would be at a higher risk of pathogenic infection, because of less aversion and disgust. This combination argues against the current opinion about pathogens dominating selective pressure in human evolution (260). The only feasible explanation would be that the longer allele of the DRD4 gene should have some immune function, protecting the ‘seeking’ carrier against pathogens.

The evidence for an immune function of the long allele of the DRD4 comes from different investigations studying the influence of the expression of dopamine receptors on innate immune cells and lymphocytes of the adaptive immune system. The various immune cells express different dopamine receptors (DRD1-DRD5) (165). The net function of dopamine receptor activation is an increase in the pro-inflammatory activity of the immune system, with the exception of the wild type DRD4 (the short allele) (261). Activation of the wild type DRD4 receptor leads to the production of the immune-suppressing cytokine IL10 (247, 254).
The long 7R allele, on the contrary, is associated with diminished cAMP production and reduced intracellular response (262). Reduced response will lead to lower immune quiescence (the normal function of wild type DRD4 (247, 263) and increase the pro-inflammatory effects of dopamine by activating other dopamine receptors (165). It is therefore conceivable that migration out of Africa selected the longer allele of the D4 dopamine receptor by inducing novelty seeking, while increasing protective inflammatory activity. Dopamine can stimulate the production of NFκB and pro-inflammatory cytokines such as TNFα and IL1, although the opposite, production of anti-inflammatory IL10, is also possible (165). This probably depends on the individual’s genotype, implying that not every individual will be capable of using the dopamine mechanism as reactivation strategy.

It seems clear that dopamine (DA) plays an important role in the immune system. Dopamine is not only produced in neurons, but also in several immune cells, including T lymphocytes (247). Dopamine seems to be recruited by the immune system to protect the host against acute infiltration by pathogens. The protective effect of dopamine signalling in the immune system against new infections is evidenced by the fact that dopamine activates resting T cells, but inhibits activated T cells (165) even in the absence of other danger signals (261). This is in line with the effects of other neurotransmitters on the immune system, increasing protection against new invaders (evolutionary beneficial) but having a negative effect on the immunological memory (264). The immunological ‘use’ of the whole body to fight infection makes sense in an evolutionary framework, considering that humans have had to fight infections as the main cause of death for thousands of generations and, as stated before, almost all humans died because of infection before the start of the 20th century.
7. **Summary and conclusion**

It can be concluded that the activated immune system puts the whole body at its disposal, by reversing the functions of metabolic hormones, organs, and even the nervous system to the energetic and pro-inflammatory benefit of the immune system (Figure 3). This response is highly protective during acute inflammation/infection and even at the start of a chronic process. The longer the inflammatory response lasts, the more it contributes to (severe) loss of lean body mass (265, 266), organ dysfunction (4), brain damage and neurodegenerative diseases (77). The protective pro-inflammatory activity of the selfish immune system is no longer beneficial once severe secondary damage to organs has been caused by the immune system.

Life would not have been possible without an immune system, and the development of complex organisms needed an even more complex immune system. The human immune system belongs to the most complex among all living organisms and serves as the blueprint for the development of antivirus software in computer programming (267). Newer systems and organs normally dominate older systems as the most basic phylogenetical law in evolution. However, this sequence may change in the face of severe or long-term danger, known as evo-devo mechanisms (268, 269). Evo-devo can reach so far back in time that inflamed lung and kidney tissue literally resembles a swim bladder (270). Chronic disease is characterised by chronic inflammation (and vice versa) and gradual loss of functions and even anatomy. It affects the whole body, including the brain. The evidence brought together in this review shows that the immune system captures a major part of energy and resources during acute inflammation, putting the whole body at its disposal. This state relates to disposal of muscles (muscle wasting), the cardiovascular system (high blood pressure, atherosclerosis), the gut (digestive problems and food intolerance) and even the brain (loss of memory and concentration in, for instance, individuals suffering from FMS). Long-term pro-inflammatory activation of the immune system would not be possible without putting the whole body at the disposal of the immune system. Because of the immune system’s capability of recruiting metabolic hormones and neurotransmitters and using them for its own benefit, it is the most selfish organ in human beings. The body at the disposal of the immune system protects the host during acute inflammation by mounting an optimal response and during chronic stress to maintain pro-inflammatory activity and to protect against possible infectious pathogens. This situation is initially protective, but becomes severely deleterious when the secondary damage to organs and tissues overrides the benefit of infectious protection. The environment in which current human beings live constantly challenges the body with multiple new metabolic signalling factors. The only organ capable of

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1 Evolutionary developmental biology, or evo-devo, broadly investigates how body plan diversity and morphological novelties have arisen and persisted in nature (132).
communicating with all organs involved in the energetic conflict because of these multiple metabolic signalling is the immune system. To prevent further conflict, the immune system takes over, using its robust power to put the whole body at its disposal and showing its selfish behaviour. This selfish behaviour of the immune system has saved hominins for millions of years. A slow-changing environment to which the immune system could gradually adapt, characterised these years. This selfish behaviour of the immune system has to be considered to be the main cause of the majority, if not all, modern diseases. The reason lies in the interaction between the evolutionary background of immune function, genetic development and notably, the current environment as the primary cause. Genes and functions are old; the environment is brand new and this conflict underlies modern disease. It should, however, be noted that the immune system is only doing what it is made for: trying to protect us.

Figure 3. The total picture of the body at the disposal of the selfish immune system.

If the immune system succeeds in doing so, the host is protected against inflammation-induced immune suppression, which would lead to cancer and possibly lethal infections (bottom right), but at the expense of the development of modern low-grade inflammatory diseases (bottom left). GLUT 1, glucose transporter 1; GLUT 4, glucose transporter 4; IS, immune system; SAM, sympathetic adrenal medular system; HPA, hypothalamus-pituitary-adrenal axis; GR, glucocorticoid receptor; BH4, tetrahydrobiopterin; IDO, indoleamine 2,3-deoxygenase; TDO, tryptophan 2,3-deoxygenase; CVD, cardiovascular diseases; FMS, fibromyalgia syndrome; CFS, chronic fatigue syndrome.
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Lifestyle and nutritional imbalances associated with Western diseases: causes and consequences of chronic systemic low-grade inflammation in an evolutionary context

Begoña Ruiz-Núñez¹, Leo Pruimboom², D.A. Janneke Dijck-Brouwer¹, Frits A.J. Muskiet¹

¹Laboratory Medicine, University Medical Center Groningen (UMCG), The Netherlands; ²University of Gerona, Faculty of Sciences, Spain and University of Graz, Unit for Life, Austria
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Abstract

In this review, we focus on lifestyle changes, especially dietary habits, that are at the basis of chronic systemic low grade inflammation, insulin resistance and Western diseases. Our sensitivity to develop insulin resistance traces back to our rapid brain growth in the past 2.5 million years. An inflammatory reaction jeopardices the high glucose needs of our brain, causing various adaptations, including insulin resistance, functional reallocation of energy-rich nutrients and changing serum lipoprotein composition. The latter aims at redistribution of lipids, modulation of the immune reaction, and active inhibition of reverse cholesterol transport for damage repair. With the advent of the agricultural and industrial revolutions, we have introduced numerous false inflammatory triggers in our lifestyle, driving us to a state of chronic systemic low grade inflammation that eventually leads to typically Western diseases via an evolutionary conserved interaction between our immune system and metabolism. The underlying triggers are an abnormal dietary composition and microbial flora, insufficient physical activity and sleep, chronic stress and environmental pollution. The disturbance of our inflammatory/anti-inflammatory balance is illustrated by dietary fatty acids and antioxidants. The current decrease in years without chronic disease is rather due to ‘nurture’ than ‘nature’, since less than 5% of the typically Western diseases are primary attributable to genetic factors. Resolution of the conflict between environment and our ancient genome might be the only effective manner for ‘healthy aging’, and to achieve this we might have to return to the lifestyle of the Paleolithic era as translated to the 21st century culture.

Keywords

Chronic systemic low grade inflammation, evolution, brain, encephalization quotient, immune system, diet, fatty acids, fish oil, fruits, vegetables, antioxidant network, metabolic syndrome, glucose, homeostasis, insulin resistance, cholesterol, lifestyle, antioxidants, resoleomics, pro-inflammatory nutrients, anti-inflammatory nutrients.

List of abbreviations

AA, arachidonic acid; ADHD, attention deficit hyperactivity disorder; AHA, American Heart Association; ALA, alpha-linolenic acid; BMI, body mass index; CARS, compensatory anti-inflammatory response syndrome; CAT, catalase; COX-2, cyclo-oxygenase-2; CRP, C-reactive protein; CVD, cardiovascular disease; DHA, docosahexaenoic acid; EBM, evidence based medicine; EBN, evidence based nutrition, EPA, eicosapentaenoic acid; EQ, encephalization quotient; FADS2, Δ-6-desaturase; FDB, familial defective apo-B100; FH, familial hypercholesterolemia; GPR120, G-protein-coupled receptor 120; GPx, glutathione peroxidase; GWAS, genome wide association studies; HDL, high density lipoprotein; HPA-axis, hypothalamus-pituitary-adrenal gland axis; HPG-axis, hypothalamus-pituitary-gonadal gland axis; HPL, human placental lactogen; HPT-axis, hypothalamic-pituitary-thyroid axis;
IGF-1, insulin-like-growth factor-1; LA, linoleic acid; LCP, long-chain polyunsaturated fatty acids; LDL, low density lipoprotein; LOX-12, lipoxygenase-12; LOX-15, lipoxygenase-15; LOX-5, lipoxygenase-5; LPS, lipopolysaccharides; LTB4, leukotrienes-B4; LX, lipoxin; NFκB, nuclear factor kappa B; NTIS, non-thyroidal illness syndrome; PGD2, prostaglandins-D2; PGE2, prostaglandins-E2; PLA2, phospholipase A2; PPAR, peroxisome proliferator activated receptor; RCT, randomized controlled trial; ROS, reactive oxygen species; RR, relative risk; SAA, serum amyloid A; SIRS, systemic inflammatory response syndrome; TNFα, tumor necrosis factor alpha; TSH, thyroid stimulating hormone; VLDL, very low density lipoprotein.

Introduction
In recent years, it has become clear that chronic systemic low grade inflammation is at the basis of many, if not all, typically Western diseases centered on the metabolic syndrome. The latter is the combination of an excessive body weight, impaired glucose homeostasis, hypertension and atherogenic dyslipidemia (the ‘deadly quartet’), that constitutes a risk for diabetes mellitus type 2, cardiovascular disease (CVD), certain cancers (breast, colorectal, pancreas), neurodegenerative diseases (e.g. Alzheimer’s disease), pregnancy complications (gestational diabetes, preeclampsia), fertility problems (polycystic ovarian syndrome) and other diseases (1). Systemic inflammation causes insulin resistance and a compensatory hyperinsulinemia that strives to keep glucose homeostasis in balance. Our glucose homeostasis ranks high in the hierarchy of energy equilibrium, but becomes ultimately compromised under continuous inflammatory conditions via glucotoxicity, lipotoxicity, or both, leading to the development of beta-cell dysfunction and eventually type 2 diabetes mellitus (2).

Insulin resistance has a bad name. The ultimate aim of this survival strategy is, however, deeply anchored in our evolution, during which our brain has grown tremendously. The goal of reduced insulin sensitivity is, among others, the reallocation of energy-rich nutrients because of an activated immune system (3, 4), limitation of the immune response, and the repair of the inflicted damage. To that end, serum lipoproteins adopt a pattern that bears resemblance with the ‘hyperlipidemia of sepsis’, accompanied by seemingly inconsistent changes in serum cholesterol, increased triglycerides, decreased HDL-cholesterol, and an increase of ‘small dense’ LDL-particles, of which the latter three constitute the triad of atherogenic dyslipidemia that is part of the metabolic syndrome (5-10).

From the perspective of our brain growth during evolution, we address the question of why homo sapiens is so sensitive to the development of insulin resistance. The purpose and the underlying mechanisms leading to insulin resistance and the associated dyslipidemia are subsequently discussed in more detail. We argue that our current Western lifestyle is the
cause of many false inflammatory triggers which successively lead to a state of chronic systemic low grade inflammation, insulin resistance, the metabolic syndrome, and eventually to the development of the above mentioned typically Western diseases of affluence. To find a solution for the underlying conflict between our environment and our ancient genome, we also go back in time. With the reconstruction of our Paleolithic diet, we might be able to obtain information on the nutritional balance that was at the basis of our genome. We argue that insight into this balance bears greater potential for healthy aging than the information from the currently reigning paradigm of ‘Evidence Based Medicine’ (EBM) and ‘randomized controlled trials’ (RCTs) with single nutrients.

**Our brain growth rendered us sensitive to glucose deficits**

*Homo sapiens* and the current chimpanzees and bonobos share a common ancestor, who lived in Africa around 6 million years ago. Since about 2.5 million years ago, our brain has strongly grown from an estimated volume of 400 mL to the current volume of approximately 1,400 mL (Figure 1). This growth was enabled by the finding of a high-quality dietary source that was easy to digest and contained an ample amount of nutrients, necessary for the building and maintenance of a larger brain. The nutritional quality of primate food correlates positively with relative brain size and inversely with body weight, suggesting that a larger brain requires a higher dietary quality (11). The necessary so-called ‘brain selective nutrients’ include, among others, iodine, selenium, iron, vitamins A and D, and the fish oil fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), that jointly are abundantly available in the land-water ecosystem. There are compelling arguments that a sizeable part of our evolution occurred at places where the land meets the water (12-15), but also that we have changed our lifestyle in a too short period of time. These changes started from the agricultural revolution (around 10,000 years ago) and became accelerated since the industrial revolution (about 100-200 years ago). They created a conflict between our current lifestyle, including our diet, and our ancient genome, that, with an average effective mutation rate of 0.5% per million years, still resides for the greater part in the Paleolithic era (16, 17). It is not by chance that the above mentioned brain selective nutrients are among those of which we currently exhibit the largest deficits worldwide. These deficits are masked by enrichment and fortification of our current diet with iodine (in salt), vitamins A and D (e.g. in margarines and milk) and iron (flour, cereals).

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2 Food quality refers to the energy content and/or the nutrient content of a diet. An increase in food quality may derive from the consumption of a diet with another composition or the modification of the diet by e.g. cooking or genetic manipulation (11).
Figure 1. Evolution of our brain size within the past 3.5 million years.
Our brain has grown fast since the homo erectus (1.7-2.0 million years ago). The newborn homo sapiens, the adult chimpanzee and the homo floresiensis (18) have brain volumes of around 400 mL. Adapted from Aiello and Wheeler (19) with permission from The University of Chicago Press.

Figure 2. Relationship between body weight and basal metabolism in 51 land mammals (20 non-primates, 30 primates, and humans).
Adapted from Leonard et al.(11) with permission from Elsevier.
Our brain consumes 20-25\(^3\) of our basal metabolism (11-17, 20) and is thereby together with the liver (19\(^3\)), our gastrointestinal tract (15\(^3\)), and skeletal musculature (15\(^3\)) among the quantitatively most important organs in energy consumption (19). The infant brain consumes as much as 74\(^\%\) of the basal metabolism (11, 21). In contrast to most other organs, the brain uses mostly glucose as an energy source. There is no other primate equipped with such a large, glucose-consuming, luxury organ as our brain. For example, our closest relative, the chimpanzees, has a brain volume of 400 mL, which consumes about 8-9\% of the basal metabolism. Because of the high energy expenditure of a large brain, it was necessary to make various adjustments in the sizes of some other organs. There is a linear relationship between body weight and basal metabolism among terrestrial mammals (Figure 2). This apparently dogmatic relationship predicts that, due to the growth of our brain, other organs with high energy consumption had to be reduced in size, what in evolution is known as a ‘trade-off’\(^4\). As a consequence of this ‘expensive tissue hypothesis’ of Aiello and Wheeler (19) our intestines, amongst others, had to become reduced in size. However, this exchange of expensive tissue probably occurred prior to, or simultaneous with, our brain growth, in which the trigger was the consumption of the easily digestible high-quality food (20) that contains the above-mentioned ‘brain selective nutrients’ from the land-water ecosystem. Under these ‘conditions of existence’ (Darwin), a single mutation in a growth regulatory gene is likely to have been sufficient for the brain to grow. This notion derives from the existence of genetically-determined micro- (22) and macrocephaly (23) and it is as a ‘proof of principle’ demonstrated by the differences in the beak lengths of Darwin’s legendary Galapagos finches (24-26). Compared with our close (vegetarian) relatives in the primate world, we possess a relatively long small intestine and a relatively short large intestine, which corresponds with the digestion of high quality food (such as meat and fish) in the small intestine, and the lesser need of a long colon for the digestion of complex carbohydrates (e.g. fiber) from a typically vegetarian diet (19). Unlike our near primates, such as the gorilla, our teeth and the attachments of our jaw muscles are not specialized for the processing of tough vegetarian food. Also our muscle mass became adapted, since its current size is relatively small compared to our body weight. For instance, when compared with the chimpanzee, we are definitely weak. On the other hand, we have a relatively sizeable fat mass, which probably serves as a guarantee for the high energy requirement of our brain.

Our brain’s energy consumption is quite stable. Unlike other organs, the energy consumption of the brain can not be downregulated at times of a negative energy balance or fasting (11, 20). Our brain also gets spared during prolonged fasting, while other organs such as the liver, spleen, kidneys and even the heart, are sacrificed for energy generation (27). This hierarchy

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3 These estimates derive from various publications and therefore do not add to 100\%. They should be regarded as indications.
4 The beneficial exchange of a certain property into another one
also applies to the prenatal brain, whose development is conserved during intrauterine growth restriction (28). An example is the Indian ‘thin fat baby’, with a birth weight of 2,700 g. Compared with its 3,500 g counterpart from the UK, this infant has a similar brain size and a relatively large fat compartment, at the expense of the somatic growth of the skeletal muscle, kidneys, liver and the pancreas (28). Our brain ranks high in the functional hierarchy and should be provided with the necessary energy at all times.

Apart from its large size, there is nothing special about our brain within the primate world. Compared with other species, primates have a very economical space-saving brain, but among the primates, brain weight correlates with the number of neurons (29-32) and intelligence (33). Actually, our brain is no more than an oversized primate brain (29). What does distinguish us from other species is the high ratio between our brain size and our body weight, which is also named encephalization quotient (EQ) (Figure 3). Toothed whales (brain weight 9,000 g) and African elephants (4,200 g) have much larger brains than humans, but they have lower EQs (34). Among the primates, EQ does not correlate with intelligence (33). Our high EQ has major implications for our energy management, particularly at times of ‘glucose shortage’. Under normal circumstances, our brain functions almost entirely on glucose, consuming up to 130 g/day (27). Compared with the apparently unlimited storage capacity for fat, we only dispose of a small reserve of glucose that is stored as glycogen in the liver (up to 100-120 g; mobilizable) and muscles (360 g; for local usage), while some glycogen can even be found in brain’s astrocytes (35). With the exception of the glycerol moiety, we cannot convert fat into glucose. The reduced carbohydrate intake that came along during evolution with the transition from vegetarians to omnivores rendered us strongly dependent on gluconeogenesis from (glucogenic) amino acids. This was possible because we simultaneously consumed more protein from meat and fish, which is also referred to as the ‘carnivore connection’ (36). After the depletion of our glycogen reserves, for instance after an overnight fast, we obtain the necessary glucose for our brain via gluconeogenesis from glycerol and amino acids. Under normal conditions, these amino acids derive from our dietary proteins after a meal, but during starvation, they become extracted from our tissues by catabolism of functional proteins, at the expense of our lean body mass. Under such circumstances of severe glucose deficit, the energetic need of our brain becomes increasingly covered by ketone bodies from fat (37, 38).
A glucose deficit leads to competition between organs for the available glucose. As previously mentioned, this occurs during fasting, but also during pregnancy and infection/inflammation. Fasting is characterized by a generalized shortage of glucose (and other macronutrients), but in case of pregnancy and inflammation we deal with active compartments competing with the brain for the available glucose, i.e. the growing child and the activated immune system, respectively. During competition between organs for glucose, we fulfill the high glucose needs of the brain by a reallocation of the energy-rich nutrients, and to that end, we need to become insulin resistant.

**Reallocation of energy-rich nutrients by insulin resistance**

The developing child grows fast in the third trimester of pregnancy. In this period, the supply of the necessary building blocks like glucose and fatty acids should be independent of the maternal metabolic status, which is known as the state of ‘accelerated starvation’ and ‘facilitated anabolism’ (38). Glucose crosses the placenta without restriction. Fetal needs are directive, since the developing fetus is high in the evolutionary hierarchy. If necessary, the fetal needs become covered at the expense of the mother, which is known as the ‘depletion syndrome’.

**Figure 3. Encephalization quotient (EQ) of selected mammals.**

*The EQ has been normalized with the cat as a reference. Data adapted from Roth and Dicke (34).*
During infection/inflammation we deal with the metabolic needs of an activated immune system for acute survival. The inactive immune system consumes about $23\%$ of our basal metabolism, of which as much as $69\%$ derives from glucose ($47\%$) and the glycogenic amino acid glutamine ($22\%$). Upon activation, the energy requirement of our immune system may increase with about $9$–$30\%$ of our basal metabolic rate. In multiple fractures, sepsis and extensive burns, we deal with increases up to $15$–$30$, $50$, and $100\%$ of our basal metabolism, respectively ($3, 4, 39$).

The way we save glucose for our brain during starvation, for the brain and the fetus during pregnancy, and for the brain and immune system during infection/inflammation, is by causing insulin resistance in selected insulin-dependent tissues. These tissues are thereby forced to switch to the burning of fat. Due to insulin resistance, the adipose tissue compartment will be encouraged to distribute free fatty acids, while the liver will be encouraged to produce glucose via gluconeogenesis and to distribute triglycerides via VLDL. The aforementioned (asymmetric) ‘thin fat baby’ with its spared brain, relatively high adipose tissue compartment, and the growth restricted body (islets of Langerhans included), has relatively high cord plasma insulin and glucose concentrations at birth ($28$). These characteristics of insulin resistance and diabetes mellitus are probably necessary for the postpartum, saving of as much as possible of the available glucose for the brain, whereas the other organs are provided with fatty acids from the sizeable adipose tissue stores. This intrauterine ‘programming’, that follows the prediction of a thrifty postnatal life comes along with health risks, notably when the prediction proves false ($40, 41$). According to the ‘Barker hypothesis’, at adult age, these children have a higher chance of diseases related to the metabolic syndrome, especially when they are raised in our current obesogenic society. The unfavorable interaction of their high EQ with a high body weight is already demonstrable at the age of $8$ years ($42$). Essentially, their postnatal risk is attributable to a (probably epigenetic) ‘intrauterine programming’, that traces back to the high hierarchical ranking of our brain in both growth and energy needs, also referred to as ‘the selfish brain’ ($43$).

Glucose intolerance ($26$) and insulin resistance have been reported in calorie restriction, extreme fasting and anorexia nervosa, and may even cause, under these circumstances, diabetes mellitus type $2$, notably in those subjects sensitive to its development ($44$). According to textbooks, insulin resistance during the third trimester of pregnancy is caused by the hormonal environment, among which HPL, progesterone, estrogens, prolactin and cortisol are mentioned. However, placental tumor necrosis factor alpha (TNFα) correlates best with measures of maternal insulin resistance ($45, 46$). Pregnancy is therefore sometimes referred to as a physiological state of systemic low grade inflammation ($47$). As a consequence of reduced insulin sensitivity, maternal circulating concentrations of energy-rich nutrients, such as glucose and fat, tend to increase, promoting their transport across the placenta.
Under non-pregnant conditions, this situation would resemble pathology, but is tolerable during the 9 months of a pregnancy, while the largest changes occur during the third trimester.

Figure 4. Mechanistic connection between inflammation and insulin resistance.

The NFκB and AP-1 Fos/June inflammatory pathways inhibit the PI3K/AKT signal transduction pathway for nutrient metabolism and the Ras/MAPK pathway for gene expression, both part of the insulin signaling. CAP, Cbl associated protein; Cbl, Proto-oncogene product; ER, endoplasmic reticulum; FFAs, Free fatty acids; Gqα/11, heterotrimeric G protein; Ikbb, I kappa B kinase Beta; IRS, insulin receptor substrate; JNK, C-jun N-terminal kinase; NFkB, nuclear factor kappa B; NO, nitric oxide; Ras/MAPK; PI3K, phosphatidylinositol 3-kinase; Ras-mitogen activated protein kinase; Shc, Src homology 2 containing protein; SOCS, suppressor of cytokine signaling; TLRs, Toll-like receptors. Adapted from de Luca and Olefsky (48) with permission from Elsevier.

During infection and inflammation, the signals for metabolic adaptation become transmitted by pro-inflammatory cytokines. The resulting insulin resistance causes reallocation of energy (i.e. the aim of the process; see above), which illustrates that inflammation and metabolism are highly integrated (49-51). At the molecular level, the interaction takes place through the influences of the nuclear factor kappa B (NFκB) and the AP-1 Fos/June inflammatory pathways on the PI3K/Akt signal transduction pathway for nutrient metabolism and the Ras/MAPK pathway for gene expression, which are both part of the insulin signal transduction (48, 52). To put it simply: the activated inflammatory signal transduction...
pathway causes inhibition of the postreceptor insulin signaling pathway, which becomes noticeable by what we know as insulin resistance (Figure 4). Insulin resistance especially refers to a grossly diminished reduction of the circulating glucose concentration by insulin. However, insulin has many functions, and thereby exerts different effects in the various organs carrying the insulin receptor. Consequently, the ‘resistance’ affects the many insulin signal transduction pathways at various degrees, and thereby works out differently with respect to the various insulin functions (1, 53). Some processes are impaired (i.e. are genuinely ‘resistant’), while others remain intact and become excessively stimulated by the compensatory hyperinsulinemia. This compensatory increase of the circulating insulin levels aims at the prevention of a disturbed glucose homeostasis and thereby the onset of type 2 diabetes mellitus. The persistence of compensatory hyperinsulinism is responsible for most, if not all, of the abnormalities that belong to the metabolic syndrome (1).

In muscle and fat cells, insulin resistance induces a diminished glucose uptake and therefore a reduced storage of glucose as glycogen and triglycerides. In fat cells, it causes decreased uptake of circulating lipids, increased hydrolysis of stored triglycerides and their mobilization as free fatty acids and glycerol. In liver cells, insulin resistance induces the inability to suppress glucose production and secretion, in addition to decreased glycogen synthesis and storage. The hereby promoted reallocation of energy-rich substrates (glucose to the brain, fetus and immune system; fat to the fetus and the organs that became insulin resistant) and the compensatory hyperinsulinemia, are meant for short-term survival, and their persistence as a chronic state are at the basis of the ultimate changes that we recognize as the symptoms of the metabolic syndrome, including the changes in glucose and lipid homeostasis (3, 4) and the increasing blood pressure. For example, the concomitant hypertension has been explained by a disbalance between the effects of insulin on renal sodium reabsorption and NO-mediated vasodilatation, in which the latter effect, but not the first, becomes compromised by insulin resistance, causing salt sensitivity and hypertension (54).

Reaven coined the term ‘metabolic syndrome’ and subsequently renamed it the ‘insulin resistance syndrome’ (1). However, it becomes increasingly clear that we could better refer to it as the ‘chronic systemic low-grade inflammation induced energy reallocation syndrome’. The reason for this broader name derives from the recognition that insulin resistance is only part of the many simultaneously occurring adaptations. To their currently known extent, these adaptations and consequences are composed of: i) reduced insulin sensitivity (glucose and lipid redistribution, hypertension), ii) increased sympathetic nervous system activity (stimulation of lipolysis, gluconeogenesis and glycogenolysis), iii) increased activity of the HPA-axis [hypothalamus-pituitary-adrenal gland (stress) axis, mild cortisol increase, gluconeogenesis, with cortisol resistance in the immune system], iv) decreased activity of the HPG-axis (hypothalamus-pituitary-gonadal gland axis; decreased androgens for
gluconeogenesis from muscle proteins, sarcopenia, androgen/estrogen disbalance, inhibition of sexual activity and reproduction), v) IGF-1 resistance (insulin-like growth factor-1; no investment in growth) and vi) the occurrence of ‘sickness behavior’ (energy-saving, sleep, anorexia, minimal activity of muscles, brain, and gut) (3).

The HPT-axis (hypothalamic-pituitary-thyroid axis) has a central role in our energy management. The adaptation of thyroid function in subjects with the metabolic syndrome is yet unclear, possibly due to the many concerted changes, such as an altered thyroid hormone binding capacity, tissue uptake, conversion of T4 into T3, and tissue-specific receptor expression and function. For example, T4 may become converted into the highly active T3 within the target cell and thereby, without visible changes of circulating hormone concentrations, bind to the intracellular thyroid hormone receptor (55). Whether intracellular T3 is converted into T3 or the inactive reverse T3 (rT3), or is used as a source of iodine to kill bacteria, depends on several factors, including cytokines, that determine the expression pattern of the three involved deiodinases (55-57). In euthyroid subjects, free T4 (FT4) is associated with insulin resistance, inversely related to total- and LDL-cholesterol, while also a positive relationship between TSH and triglycerides has been documented (58). The reported changes during metabolic syndrome (59), low-grade inflammation and insulin resistance (60) are inconsistent, but do bear great resemblance with subclinical hypothyroidism, with high-normal or slightly elevated TSH, and normal FT4 concentrations (61, 62). Insulin resistance has recently been associated with an increased T3/rT3 ratio, which is a measure of peripheral thyroid hormone metabolism and suggests increased thyroid hormone activity (63). In contrast, during fasting, energy expenditure becomes downregulated, resulting in a normal or decreased TSH and decreased serum thyroid hormone concentrations (64).

Downregulation of the HPT-axis with reductions of T3, T4 and TSH, and an increase of rT3 (and thus a decrease of the T3/rT3 ratio) occurs progressively with the severity of the ‘non-thyroidal illness syndrome’ (NTIS, also called the ‘Low T3 syndrome’ and ‘euthyroid sick syndrome’) (55) which is explained as an adaptation of the body to prevent excessive (protein) catabolism as part of the acute phase response (56).

All of the above mentioned adaptations of our metabolism are associated with changes in the serum lipoprotein profile, which are part of the metabolic syndrome. The purpose of these changes will be explored in more detail below.

**Changes in serum lipoproteins**

The quantitative and qualitative changes in the composition of serum lipoproteins resulting from an inflammatory trigger have, in addition to the reallocation of energy-rich nutrients (fatty acids to the insulin resistant organs), at least two other goals (5-10, 65). These are: i) the
modulation of the immune response by which we protect ourselves from the harmful effects of invading bacteria, viruses and parasites, and ii) the restoration of the hereby inflicted damage. However, if the subsequent changes in structure and function of lipoproteins persist, they contribute to the development of atherosclerosis (66). These long term complications have not exerted selection pressure during evolution and, consequently, no solution has come into existence via the habitual process of spontaneous mutation and natural selection.

The inflammatory trigger during an infection with Gram-negative bacteria is initiated by lipopolysaccharides (LPS). Circulating lipoproteins aid in the clearance of this LPS. Hence, lipoproteins do not only have functions in transporting lipids to and from tissues, but also play important roles in limiting the inflammatory response (67). The ability of lipoproteins to bind LPS is proportional to the cholesterol content of the lipoprotein (68), but the phospholipids/cholesterol ratio of the lipoprotein is the principal determinant of the LPS-binding capacity (69). The available phospholipid surface is thus of special importance and is, under normal circumstances, the largest for the circulating HDL. However, critically ill patients exhibit decreases of both esterified cholesterol and HDL (see below) and in those patients, LPS is mainly taken up in the phospholipid layers of LDL and VLDL. Binding of LPS to lipoproteins prevents activation of LPS-responsive cells and encourages LPS clearance via the liver to the bile. In line with this mechanism, it has been observed that a decrease in plasma lipoproteins in experimental models increases LPS-induced lethality (69).

The protective role of LDL is already known for some time, and this process has probably been exploited during evolution. Currently, there are over one thousand LDL-receptor mutations, many of which lead to a reduced or absent hepatic uptake of LDL particles, and consequently, to an elevated serum LDL-cholesterol (70). The carriers of these mutations have ‘familial hypercholesterolemia’ (FH; incidence about 1/400 in The Netherlands) or ‘defective apo-B100’ (FDB), if the mutation is located in the LDL-receptor ligand. They constitute autosomal dominant disorders with a high risk of premature atherosclerosis and mortality from CVD (71). The arising question is why evolution has preserved so many apparently detrimental mutations in the LDL-receptor. Research with data from the population registry office in The Netherlands showed that subjects with FH lived longer until 1800, which turned into a shorter lifespan than the general population after 1800 (72). Important support for an explanation came from studies with LDL-receptor knockout mice, and also with transgenic mice overexpressing apo-AI, the structural protein of HDL. These mutants have a high LDL- and HDL-cholesterol, respectively, are resistant to LPS-induced mortality, and have better survival of severe Gram-negative infection compared with the wild type (66, 73). In other words, FH might have become widespread during evolution due to the modulating effect of a high LDL (i.e. ‘a high cholesterol’) during Gram-negative infections, that were much more common in the past. This benefit might have become a risk following the introduction of a
typically Western lifestyle (see below), to which subjects with FH seem particularly sensitive (72).

**Figure 5. Changes in reverse cholesterol transport during the acute phase response.**

Lipopolysaccharides (LPS) and cytokines reduce the ABCA1 (ATP binding cassette transporter A1) and the cholesterol efflux from peripheral cells to HDL. LPS reduces the activities of various proteins involved in HDL metabolism, such as lecithin-cholesterol acyltransferase (LCAT), cholesterol ester transfer protein (CETP) and hepatic lipase (HL). LPS and cytokines also down-regulate hepatic scavenger receptor class B type 1 (SRB1), resulting in a decreased cholesterol ester (CE) uptake in the liver. FC, free cholesterol; LDL-R, LDL receptor; LRP, LDL receptor-related protein; PLTP, phospholipid transfer protein. Adapted from Khovidhunkit et al. (66) with permission from The American Society for Biochemistry and Molecular Biology.

As mentioned above, among the lipoproteins, notably HDL has the capacity to bind LPS and thereby to prevent an LPS-induced activation of monocytes and the subsequent secretion of proinflammatory cytokines (5). However, during the ‘lipidemia of sepsis’, the HDL concentration decreases while also the HDL particles decrease in size (6). Their function changes as part of the acute phase response: the immunomodulatory properties vanish to a high extent and HDL even becomes proinflammatory. The apo-A1 and cholesterol esters are lost from the HDL particle, the activities of HDL-associated enzymes and exchange proteins decrease, and these proteins are, among others, replaced by serum amyloid A (SAA) (5, 6). Like CRP, SAA is produced in the liver as part of the acute phase response. SAA is 90% located in HDL, prevents the uptake of cholesterol by the liver and directs it to other cells such as macrophages (8, 66). Both the decreasing HDL-cholesterol and the concomitantly reduced ‘cholesterol reverse transport’, promote the accumulation of cholesterol in the tissues, where it is needed for the synthesis of steroid hormones (e.g. cortisol) in the adrenal glands, the immune system and for the synthesis of cellular membranes that became damaged by the infection (66). Also the formation of small dense LDL (74) might be functional because these
particles are poorly cleared by the LDL-receptor, easily penetrate the subendothelial space and by their binding to the subendothelial matrix, take their cholesterol cargo to the sites of damage in a highly efficient manner. It appears that there are numerous mechanisms that jointly cause the active inhibition of the reverse cholesterol transport in response to an acute phase response (Figure 5) (66, 75).

Summarizing thus far, we humans are extremely sensitive to glucose deficits, because our large brain functions mainly on glucose. During starvation, pregnancy and infection/inflammation, we become insulin resistant, along with many other adaptations. The goal is the reallocation of energy-rich substrates to spare glucose for the brain, the rapidly growing infant during the third trimester of pregnancy, and our activated immune system that also functions mainly on glucose. Under these conditions, the insulin resistant tissues are supplied with fatty acids. Other goals of the changes in the serum lipoprotein composition are their role in the modulation of the immune response by the clearance of LPS during infection/inflammation and the redirection of cholesterol to tissues for local damage repair. The metabolic adaptations caused by inflammation illustrate the intimate relationship between our immune system and metabolism. This relation is designed for the short term. In a chronic state it eventually causes the metabolic syndrome and its sequelae. We are ourselves the cause of the chronicity. Our current Western lifestyle contains many false inflammatory triggers and is also characterized by a lack of inflammation suppressing factors. These will be described in more detail below.

Lifestyle-induced chronic systemic low grade inflammation
An inflammatory reaction is the reflection of an activated immune system that aims to protect us from invading pathogens or reacts to a sterile infection. If an activated immune system is uncontrolled, the resulting secondary reactions have the ability to kill us. Rogers (76) expresses it as follows: ‘...inflammation may be useful when controlled, but deadly when it is not. For example, head trauma may kill hundreds of thousands of neurons, but the secondary inflammatory response to head trauma may kill millions of neurons or the patient’. It is clear that an inflammatory reaction that has started should subsequently be ended.
Table 1. Environmental factors that may cause chronic systemic low grade inflammation.

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<td>'Healthy obesity'</td>
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<td>'Sick building syndrome'</td>
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<td>Atmospheric CO2</td>
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Adapted from Egger and Dixon (77).

There are many factors in our current Western lifestyle that jointly cause a state of chronic systemic low grade inflammation, which in turn leads to chronically compromised insulin sensitivity, compensatory hyperinsulinemia and, eventually, the diseases related to the metabolic syndrome. Lifestyle factors that cause inflammation can be subdivided into an unbalanced composition of the diet (usually referred to as ‘malnutrition’) (78-80) and non-food related factors (77), which partly exert their influence via obesity (81) (Table 1). Among the pro-inflammatory factors in our current diet, we find: the consumption of saturated fatty acids (82) and industrially produced trans fatty acids (83, 84), a high ω6/ω3 fatty acid ratio (85-87), a low intake of long-chain polyunsaturated fatty acids (LCP) of the ω3 series (LCPω3) from fish (88, 89), a low status of vitamin D (90-92), vitamin K (93) and magnesium (94-96), the ‘endotoxemia’ of a high-fat low-fiber diet (97, 98), the consumption of carbohydrates with a high glycemic index and a diet with a high glycemic load (99, 100), a disbalance between the many micronutrients that make up our antioxidant/pro-oxidant network (101-103), and a low intake of fruit and vegetables (103, 104). The ‘dietary inflammation index’ of the University of North Carolina is composed of 42 anti- and proinflammatory food products and nutrients. In
this index, a magnesium deficit scores high in the list of pro-inflammatory stimuli (105). Magnesium has many functions, some of them, not surprisingly, related to our energy metabolism and immune system, e.g., it is the cation most intimately connected to ATP (95). Indirect diet-related factors are an abnormal composition of the bacterial flora in the mouth (106), gut (106, 107), and gingivae (108-110). Chronic stress (111, 112), (passive) smoking and environmental pollution (77), insufficient physical activity (113-118) and insufficient sleep (119-123) are also involved.

All of the above listed lifestyle factors exhibit interaction and are therefore difficult to study in isolation. As an example, the bacterial flora may change secondary to the composition of our diet. An inflammatory reaction might be at the basis of the observed relation between the abnormal bacterial species in both our oral cavity and intestine and our serum HDL- and LDL-cholesterol (106). Saturated fats may cause an inflammatory reaction especially when they are combined with a carbohydrate-rich diet, notably carbohydrates with a high glycemic index, and especially in subjects with the insulin resistance syndrome (124-128).

**Mechanisms of lifestyle-induced inflammation**

Diets high in refined starches, sugar, saturated and trans fats, and low in LCPω3, natural antioxidants, and fiber from fruits and vegetables, have been shown to promote inflammation (82-84, 129-131) (Table 1). As most chronic (inflammatory) diseases have been linked to diet, modifying it could prevent, delay or even heal these diseases. Obviously, inflammation is an essential process for survival, but our immune system should be carefully controlled to limit the unavoidably associated collateral damage (132). For instance, wound healing and other immune challenges become controlled in our body by a process coined by Serhan et al. (133-135) as *resoleomics*, using metabolites produced from the LCP arachidonic acid (AA), EPA and DHA (85, 133-136). However, our inflammatory and resolution genes operate nowadays in a completely different environment than the one to which they became adapted through mutation and natural selection. In most (if not all) chronic diseases typical of Western societies, the inflammatory response is not concluded because of suboptimal or supramaximal responses (137, 138).

It has been estimated that 10% of all deaths in the Netherlands are attributable to unfavorable dietary composition and 5% to overweight. In this scenario, the major contributors to diet-associated death were insufficient intakes of fish, vegetables and fruits, with less important roles for too high intakes of saturated and trans fatty acids (139). The consumption of fish, fruit and vegetables is considered too low in most Western countries (139-143). In the USA, low dietary ω3 fatty acids and high dietary trans fatty acids may have accounted for up to 84,000 and 82,000 deaths, respectively, in 2005, while a low intake of fruit and vegetables
might have been responsible for 58,000 deaths (144). The Dutch (145) and the American Heart Association (AHA) (146) dietary guidelines recommend to consume at least two servings of fish per week (particularly fatty fish), but in 1998, the average fish consumption in The Netherlands amounted to hardly 3 times per month (139). Only about 7% of the 9-13 year-old Dutch children eat fish twice or more per week and 10% never eat fish (147). In the USA, the estimated intake of fish in 2007 was about 0.7 kg per month, per person. More preoccupying is the fact that the USA is considered the third largest consumer of seafood in the world (148, 149). Despite improvements of the fatty acid contents of food products, only 5% of the Dutch population follows a diet with the recommended fatty acid pattern (139). Eating fish once weekly was associated with a 15% lower risk of CVD death compared with a consumption of less than once per month (150), while each 20 g/day increase in fish consumption was related to a 7% lower risk of CVD mortality (151).

The current Dutch recommendation for adults is 200 g fruits and 200 g vegetables per day (139), while in the USA, 4-5 servings of fruits and 4-5 servings of vegetables are recommended in a 2,000 kcal diet (152). Between 1988 and 1998, the consumption of fruit and vegetables in The Netherlands declined 15–20% and currently, less than 25% of the Dutch population follows the recommendations regarding the consumption of fruit, vegetables and dietary fiber (139). As an example, currently 99% and 95% of the 9-13 year old Dutch do not adhere to the advice of consuming 150 g/day vegetables and 200 g/day fruits, respectively (147). Meta analyses of prospective studies indicated that <3 vs. >5 servings of fruits and vegetable per day correspond with a 17% reduction in coronary heart disease (153) and 26% reduction in stroke (154), while the relation of low intakes with mouth, pharynx, esophagus, lung, stomach, colon and rectum cancer is considered substantially convincing (155).

In view of the numerous nutrients present in our food and their many mechanisms of action in the inflammatory response, we selected two nutrient classes, i.e. the LCP from fish (LCPω3; notably EPA and DHA), and the antioxidants in fruit and vegetables, to illustrate the many dietary components involved in our pro-inflammatory/anti-inflammatory balance. However, before embarking into these nutrient classes, it should be emphasized that our food is in reality composed of biological systems, such as meat, fish, vegetables and fruits, in which nutrients obey to the balance that comes along with living material. Therefore, focusing on specific, presently known mechanisms without sufficient knowledge of the many possible interactions between the numerous nutrients in our food should be regarded as a serious limitation. This is a reductionist approach, whereas system dynamics and holistic approximations would be more appropriate.
Fatty acids and inflammation

The media are consistently reporting on advises to reduce fat consumption to avoid risks associated with obesity, CVD, diabetes and other chronic diseases and conditions. Among the macronutrients, fat does indeed contain the highest amount of energy per gram. However, from a thermodynamic point of view, a ‘calorie is a calorie’ (156), implying that any macronutrient consumed in disbalance with energy expenditure and thermogenesis might cause obesity. A recent in-depth study revealed that ‘a calorie is not a calorie’ in a metabolic sense, showing that isocaloric diets with different macronutrient compositions have different effects on resting and total energy expenditure with decreasing energy expenditures in the sequence low-fat diet<low-glycemic diet<very low-carbohydrate diet (157), and thereby suggesting that the diet with the highest protein and fat content gives rise to the lowest weight gain. However, whether the intake of fat per se and, as a matter of fact, any isolated nutrient (158), can be held responsible for the epidemics of obesity, remains controversial and counter intuitive (159-161). Moreover, it is becoming increasingly clear that about 10-25% of obese subjects have little CVD and type 2 diabetes mellitus risk (a condition coined ‘healthy obesity’) (162, 163), that lean physically unfit subjects have higher risk of CVD mortality than obese, but fit, subjects (164), and that it is the quality and not the quantity of fat that conveys a major health hazard (165). The type of dietary fat affects vital functions of the cell and its ability to resist disfunction e.g. by influencing the interaction with receptors, by determining basic membrane characteristics and by producing highly active lipid mediators (166, 167).

Saturated fat intake has been associated with inflammation (168, 169). However, the widely promoted reduction of saturated fatty acids is increasingly criticized (170) and also the AHA advisory to replace saturated fatty acids in favor of linoleic acid (LA) to 5-10 en% (171). Insufficient intake of particular fatty acids is, on the other hand, likely to contribute to health hazards, including increased risk of infection (172), dysregulated chronobiological activity and impaired cognitive and sensory functions (especially in infants) (173). Among these important fatty acids are the LCPω3 derived from fish, of which EPA and DHA are the most important members. In 2003, the intake of EPA+DHA by adults in The Netherlands amounted to approximately 90 mg/day (women 84 mg/day and men 103 mg/day) (174), while the recommendation is 450 mg/day (175). This recommendation is based on an optimal effect in preventing CVD (anti-arrhythmic effect), but there is good evidence that higher intakes may convey additional favorable effects because of their anti-thrombotic properties and their ability to reduce blood pressure, heart rate and triglyceride levels (131). It was calculated that our Paleolithic ancestors living in the water-land ecosystem had daily intakes of 6-14 g EPA+DHA (176), which correspond with the intakes by traditionally living Greenland Eskimos (177), who, because of their low incidence of CVD, were at the basis of the research on the beneficial effects of fish oil that started in the seventies (178-180).
Both EPA and DHA must be in balance with AA, which is the major LCPω6 derived from meat, poultry, eggs (181-183) and also lean fish (184, 185). Each of these LCP may be synthesized by desaturation, chain elongation and chain shortening from the parent ‘essential fatty acids’ LA (converted to AA) and alpha-linolenic acid (ALA) (converted to EPA and DHA) (186), even though the production of EPA, and notably DHA, occurs with difficulty in humans (187). Included among the symptoms of LA, LCPω3 and LCPω6 deficiencies are fatigue, dermatological problems, immune problems, weakness, gastrointestinal disorders, heart and circulatory problems, growth retardation, development or aggravation of breast and prostate cancer, rheumatoid arthritis, asthma, preeclampsia, depression, schizophrenia and ADHD (173, 188-190).

LCPω3 are implicated in many diseases and conditions, including CVD, psychiatric diseases, pregnancy complications and suboptimal (neuro) development (86, 191-196). Moreover, a growing number of studies indicate the protective effects of dietary LCPω3 on mood symptoms, cognitive decline, depression (197, 198), Alzheimer’s disease (199) and, more generally, impaired quality of life both in the elderly (200, 201) and younger (202) populations.

LCPω3 are involved in numerous processes including energy generation, growth, cell division, transfer of oxygen from the air to the bloodstream, hemoglobin synthesis, normal nerve impulse transmission and brain function. Many different mechanisms are operational: LCPω3 mediate potent anti-inflammatory and insulin sensitizing effects through their interaction with a membrane receptor named G-protein-coupled receptor 120 (GPR120) (203, 204); they act at the gene expressional level by binding to nuclear receptors, such as the peroxisome proliferator activated receptors (PPARs) (205-207); and they modulate physical and metabolic properties of membranes through their incorporation into phospholipids and thereby impact on the formation of lipid rafts (134, 208, 209). Important common denominators in each of these interactions seem to be their anti-inflammatory and metabolic effects, again illustrating the intimate connection between the immune system and metabolism (50, 51).

The modernization of food manufacturing, preservation processes and food choices have dramatically altered the balance between LCPω3 and LCPω6 in the Western diet, notably by increasing the intake of LA from refined vegetable oils and a concomitant decrease in the intake of LCPω3 from fish (210, 211). It is gaining acceptance that it is not the amount of fat but the balance between the different types of fatty acids that is important (211, 212). A high ω6/ω3 fatty acid ratio has been demonstrated to have an inflammatory effect (86, 212, 213), while a higher intake of LCPω3 in the form of EPA and DHA regulates the production of inflammatory and resolving cytokines and decreases LA levels in both plasma phospholipids.
and cell membranes (183, 214). The conversions of LA and ALA to AA and to EPA+DHA, respectively, depend on the same enzymes in the desaturase and elongase cascade, with Δ6-desaturase (FADS2) as a rate-limiting enzyme (215) that functions twice in the biosynthesis of DHA (216). Increased consumption of ALA gives rise to an increased ALA/LA ratio and EPA+DHA content in cell membranes that comes together with a reduction of the AA content (216, 217), and thereby influences the balance between inflammation and its subsequent resolution (Figure 6) (218-220). Conversely, a higher LA level in plasma phospholipids and cell membranes emerges as a major factor responsible for incomplete resoleomics reactions and the associated immune paralysis (214, 220, 221) (Figure 6), which is attributed to the competitive inhibition of LA in the conversion of ALA to EPA and DHA and also to the competition of LA in the incorporation of EPA and DHA into cellular phospholipids (183, 214, 216).

**Figure 6.** LCPω6 and LCPω3 postulated involvement in the inflammatory reaction in sepsis and its subsequent resolution.

Sepsis causes a systemic inflammatory response giving rise to the ‘systemic inflammatory response syndrome’ (SIRS). The inflammatory response is followed by a compensatory anti-inflammatory response, which results in the ‘e’ (CARS), characterized by a weakened host defense and augmented susceptibility to secondary infections. An inflammatory response should not only be initiated, but also stopped to limit collateral damage produced by the immune system and to prevent immune paralysis. LCPω6 (AA) are involved in the initiation of the inflammatory reaction, while LCPω3 (EPA and DHA) are involved in its resolution (see also Figure 7). a) A high LCPω6/LCPω3 ratio, e.g. low fish intake, intensifies the SIRS reaching a state of hyper-inflammation, while the CARS leads to a state of immune paralysis. b) A low LCPω6/LCPω3 ratio dampens both the SIRS and CARS, resulting in a more balanced immune response and preventing hyper-inflammation and immune-paralysis. SIRS, systemic inflammatory response syndrome; CARS, compensatory anti-inflammatory response syndrome. Adapted from Mayer et al. (220) with permission from Wolters Kluwer Health.

LCPω3 and LCPω6 have distinct functions in the inflammatory reaction and its resolution. In the first phase of the inflammatory process, the pro-inflammatory eicosanoids leukotrienes-
B4 (LTB4) and prostaglandins-E2 and D2 (PGE2 and PGD2) (222, 223) are generated by macrophages from their precursor AA with the help of the lipid-oxidizing enzyme lipoxygenase-5 (LOX-5) and cyclo-oxygenase-2 (COX-2) (224-226). At the same time, PGE2 and/or PGD2, although initially pro-inflammatory, determine the switch to the next phase: the resolution of the inflammation (227) via the so-called ‘eicosanoid-switch’. The production of the LOX-5 enzyme becomes limited, while anti-inflammatory lipoxins (LXs) are produced from AA through the activation of lipoxygenase-12 (LOX-12), lipoxygenase-15 (LOX-15) and acetylated COX-2 (228). At the site of inflammation, LOX-12 produced by platelets converts LTA4 to LXA4 and LXB4. Along with AA, both LOX-12 and -15 are involved in the biosynthesis of specialized bioactive lipid mediators, coined resolvins, (neuro)protectins (135) and maresins (229), which derive from EPA and DHA (Figure 7) (85, 134, 172). Several studies have illustrated the involvement of these lipid mediators in vascular inflammation and atherosclerosis (85, 228, 230, 231). They possess potent anti-inflammatory and pro-resolving actions that stimulate the resolution of acute inflammation by reducing and/or limiting the production of a large proportion of the pro-inflammatory cytokines produced by macrophages. Furthermore, LXA4, protectin D1 and resolvin D1 stimulate the phagocytic activity of macrophages toward apoptotic cells and inhibit inflammatory cell recruitment (232, 233) thereby protecting tissues from excessive damage by the oxidative stress that comes along with immune defense mechanisms and others. By their inhibitory actions on the recruitment of inflammatory cells, they allow the resolution phase to set in (234) and finish the inflammatory process with the return to homeostasis (136, 227).

Accordingly, LCPω3 given at doses of hundreds of milligrams to grams per day, exhibits beneficial actions in many inflammatory diseases (88, 190, 194, 235, 236). For example, DHA has been shown to suppress NFκB activation and COX-2 expression in a macrophage cell line (168, 237). Different studies demonstrated the nutrigenetic modulation of the 12/15-LOX by providing endogenous anti-inflammatory signals and protection during the progression of atherogenesis (231, 238, 239), which seem to be totally annulled in the presence of Western diet induced hyperlipidemia. As some eicosanoids regulate the production of inflammatory cytokines (85, 134, 135) an LCPω3-induced decrease in pro-inflammatory eicosanoid production might affect the production of pro-inflammatory cytokines. Equally important is the observation that LCPω3 also modulate the activation of transcription factors involved in the expression of inflammatory genes (e.g. NFκB, phosphatidylinositol 3-kinase (PI3K)) (240). Hence, a high fish consumption, and especially fatty fish, rich in EPA and DHA, seems of crucial importance in the primary and secondary prevention of (Western) chronic diseases (241, 242), although it should be emphasized that fish is not a synonym of fish oil and also that insufficient fish consumption is certainly not the only factor involved in the pro-inflammatory Western lifestyle (Table 1).
Figure 7. Biosynthesis of inflammatory and resolving lipid mediators.

AA is released from membrane phospholipids by phospholipase A₂ (PLA₂) and metabolized by COXs or 5-LO to form inflammatory mediators, such as prostaglandins and leukotrienes. During the process of resolution, there is a ‘switch’ from the biosynthesis of inflammatory mediators to the formation of lipid derivatives with anti-inflammatory and pro-resolving properties, including lipoxins and 15-d-PGJ₂. EPA and DHA are converted to potent anti-inflammatory and pro-resolving lipid mediators like resolvins (E1 and D1) and protectins. ASA, acetylsalicylic acid, CYP450, cytochrome P450, COX-1, cyclooxygenase-1, COX-2, cyclooxygenase-2; 5-LO, 5-lipoxygenase; 12-LO, 12-lipoxygenase; 15-LO, 15-lipoxygenase; PGE₂, prostaglandin-E₂; PGD₂, prostaglandin-D₂; LTs, leukotrienes; 15d-PGJ₂, 15-deoxy-delta-12,14-prostaglandin J₂; 15-epi-LXA₄, 15-epi-lipoxin A₄; LXA₄, lipoxin A₄. Adapted from González-Pérez and Clària (243) with permission.

Role of the antioxidant network

The largest contributor to mortality and morbidity worldwide is age-related, non-communicable disease, including cancer, CVD, neurodegenerative diseases and diabetes (244). Even though these are multi-factorial diseases with many pathophysiological mechanisms, a common finding is oxidation-induced damage through oxidative stress (245, 246). Appropriate antioxidant intake has been proposed as a solution to counteract the deleterious effects of reactive oxygen species (ROS; e.g. hydrogen peroxide, hypochlorite anion, superoxide anion and hydroxyl radical), with substantial evidence upholding the contention that: a diet rich in natural antioxidants supports health (104, 246), is associated with lower oxidative stress and inflammation (77, 103, 140), and is therefore associated with lower risk of cancer, CVD, Alzheimer’s disease, cataracts, and some of the functional declines associated with aging (247–251).
Molecular oxygen is essential to aerobic life and, at the same time, an oxidizing agent, meaning that it can gain electrons from various sources that thereby become ‘oxidized’, while oxygen itself becomes ‘reduced’ (252, 253). In general terms, an antioxidant is ‘anything that can prevent or inhibit oxidation’ and these are therefore needed in all biological systems exposed to oxygen (252). The emergence of oxygenic photosynthesis and subsequent changes in atmospheric environment (254) forced organisms to develop protective mechanisms against oxygen’s toxic effects (255). Change is implicit to evolution and evolution results in adaptation to change (256). As a result, many enzymatic reactions central to anoxic metabolism were effectively replaced in aerobic organisms and antioxidant defense mechanisms evolved (257, 258). The continuous exposure to free radicals from a variety of sources led organisms to develop a series of systems (259) acting as a balanced and coordinated network where each one relies on the action of the others (260, 261).

Oxidative stress occurs when there is a change in this balance in favor of ROS (262) that may occur under several circumstances, ranging from malnutrition to disease (263, 264). Damage by oxidation of lipids (262, 265, 266), nucleic acids and proteins changes the structure and function of key cellular constituents resulting in the activation of the NFκB pathway, promoting inflammation, mutation, cell damage and even death (252, 260, 267), and is thereby believed to underlie the deleterious changes in aging and age-related diseases (102, 244). The prevention and/or inhibition of oxidation can be achieved by several types of specialized antioxidant mechanisms depicted in Table 2 (260). Our antioxidant system is composed of two networks (Figure 8), namely, the antioxidant network of non-enzymatic antioxidants that we obtain mostly via the diet (268), and the antioxidant enzymes that we synthesize ourselves and that carry metal ions for their appropriate functioning in ROS clearance. Members of the non-enzymatic antioxidants are e.g. ascorbic acid (vitamin C), alpha-tocopherol (vitamin E), carotenoids, and the polyphenols (269, 270). For instance, quercetin, one of the most common flavonoids in the human diet, and resveratrol, a well-known stilbenoid present mostly in berries and the skin of red grapes, have demonstrated favorable effects on glucose metabolism by attenuating TNFα-mediated inflammation and insulin resistance in primary human adipocytes (271). Typical examples of the antioxidant enzymes are superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) (252).
Table 2. Types of antioxidant action.

<table>
<thead>
<tr>
<th>Action</th>
<th>Examples</th>
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<tr>
<td>Prevention</td>
<td>Protein binding/inactivation of metal ions</td>
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<td></td>
<td>Transferrin, ferritin, ceruloplasmin, albumin</td>
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<tr>
<td>Enzymatic</td>
<td>Specific channelling of ROS into harmless</td>
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<tr>
<td>Neutralization</td>
<td>products</td>
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<td></td>
<td>SOD, catalase, glutathione peroxidase</td>
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<tr>
<td>Scavenging</td>
<td>Sacrificial interaction with ROS by expendable</td>
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<td></td>
<td>(recyclable or replaceable) substrates</td>
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<tr>
<td></td>
<td>Ascorbic acid, alpha tocopherol, uric acid,</td>
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<tr>
<td></td>
<td>glutathione</td>
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<tr>
<td>Quenching</td>
<td>Absorption of electrons and/or energy</td>
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<td></td>
<td>α-tocopherol, β-carotene, astaxanthin</td>
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ROS, reactive oxygen species; SOD, superoxide dismutase. Adapted from Benzie (260).

While the prevention of oxidative stress by enhancing the antioxidant defense mechanisms may diminish the production of inflammatory mediators and thereby slow aging and lower risk of certain diseases (102, 245, 249), it should at the same time be appreciated that ROS also exert essential metabolic and immune functions. For example, oxidative phosphorylation is based on electron transport (272), which renders free radicals’ inevitable byproducts of mitochondrial metabolism (273). Mitochondrial oxidants may function as signaling molecules in the communication between the mitochondria and the cytosol (273), while TNFα-induced apoptosis may involve mitochondria-derived ROS (274). The innate immune system kills microbes by means of the respiratory burst (275). A certain level of ROS may also be essential to trigger antioxidant responses (276). Repeated exposure to sublethal stress has been proposed to result in enhanced stress resistance and increased survival rates, which in the dose–response curve is better known as hormesis (277). Intracellular ROS may stimulate gene expression of antioxidant and immunoreactive proteins (278), while SOD may become upregulated in chronic exercise through the binding of NFκB to the SOD promoter (279, 280).

Consequently, certain antioxidants may inhibit mitochondrial biogenesis, interfere with the horneric effects of ROS (281, 282) or have other adverse effects. Effective prevention of ROS formation and their removal may therefore upset energy metabolism, cell signaling pathways and the immune system, and thereby paradoxically increase the risk of chronic disease (283). Moreover, any antioxidant is also a potential pro-oxidant because in its scavenging action it gains an extra electron that can initiate a new radical reaction when transferred to an acceptor, either spontaneously or upon decomposition (284, 285). Possibly through its
prooxidant action or other mechanisms (286), meta-analyses of studies with β-carotene dosages above 20 mg/day have shown increased risk of lung cancer in the total population, smokers and asbestos workers; and of stomach cancer in smokers and asbestos workers (287). Analogously, oral antioxidants to limit muscle damage following exercise training may be detrimental to health and performance (288), while β-carotene, vitamin A and vitamin E supplements have been connected with higher risk of all-cause mortality (289), although the outcome of the latter meta-analysis has been contested (290). Moreover, not all antioxidants are created equal. Astaxanthin, a carotenoid from the land-water ecosystem, does not appear to exhibit pro-oxidant properties (291) when supplemented alone, even at high doses (292), and has been shown to decrease oxidative stress and inflammation in various circumstances (266, 293).

Chronic inflammation results in the chronic generation of free radicals, which may cause collateral damage and stimulate signaling and transcription factors associated with chronic diseases (294, 295). The hypothesis that dietary antioxidants lower the risk of chronic diseases has been developed from epidemiological studies consistently showing that consumption of fruit and vegetables is strongly associated with a reduced risk of these diseases (104, 248, 250). Regular consumption of green tea (296) and red wine (103, 297), both rich in polyphenols, decreases DNA damage, and the same holds for the kiwifruit (298) and watercress (299), both harboring high amounts of carotenoids and vitamin C. On a calorie basis, fruits and vegetables are not only richer in many vitamins and minerals, when compared with cereals, meat or fish, but also in antioxidants (300). These may collectively be responsible of the aforementioned protection of fruits and vegetables in chronic diseases, including CVD (248) and cancer (249). Plants harbor similar defense mechanisms as animals for protection against ROS (301). Some of their antioxidants are part of their arsenal of ‘secondary metabolites’, defined as those organic compounds that are not directly involved in normal growth, development and reproduction, but in long term survival and fecundity (302). The plant secondary metabolites are largely involved in the chemical defense against herbivores, microbes, viruses and competing plants, in signaling and in nitrogen storage (303); and some (e.g. polyphenols, carotenoids) also serve functions in the protection against ROS. The underlying metabolic pathways towards secondary metabolites lead to a series of related compounds that are usually composed of few major metabolites and several minor components differing in the position of their functional groups (303). Animals consuming fruits and vegetables may employ these plant secondary metabolite networks for their own purposes, including maintenance of inflammatory/anti-inflammatory balance, cancer chemoprevention and protection against ROS (303).
Figure 8. Antioxidant defense mechanisms. 

An overview of the antioxidant system present in the human body. Various types of antioxidant systems have developed through time, reflecting different selection pressures. Different forms have developed for the same purpose, for example, SODs, peroxidases and GPx are important members of the antioxidant enzyme capacity group. Tocopherols and ascorbic acid, as representatives of the antioxidant network, are manufactured only in plants, but are needed by animals. Ascorbic acid is an essential antioxidant, but cannot be synthesized by homo sapiens. In humans, therefore, antioxidant defense against toxic oxygen intermediates comprises an intricate network which is heavily influenced by nutrition. GR, glutathione reductase; GSG, reduced glutathione; GSH-Px, glutathione peroxidase; GSSG, oxidized glutathione; GST, glutathione-S-transferase; MSR, methionine sulfoxide reductase; PUFA, polyunsaturated fatty acids; S-AA, sulphur amino-acids; SH-proteins, sulphydryl proteins; SOD, superoxide dismutase; Fe Cu, transition metal-catalysed oxidant damage to biomolecules. Adapted from Strain (304) with permission from Cambridge University Press.

In view of the yet poorly understood complex antioxidant networks composed of many compounds, it seems improbable to find a single 'magic bullet' to prevent and treat chronic diseases associated with ROS. Protective effects of fruits and vegetables may originate from their numerous phytochemicals working in concert (305) and from many different mechanisms of action that are not solely related to ROS. A purified phytochemical may not have the same health benefit as that phytochemical present in whole foods or a mixture of foods (250, 306). In biological systems, toxins may become nutrients, while nutrients may become toxic in other situations (268), for example when disbalanced with other nutrients. Rather than translating our food into an assembly of nutrients where each has to prove its
health benefits by scientific means, the objective should be to embrace a eucaloric diet that provides the adequate amount of nutrients from whole foods to maintain our body homeostasis. ‘Adequacy’ may in this sense be translated into causing an optimal interaction between our diet (and our lifestyle in general) with our genome, that is: nurture in balance with nature.

**Evolutionary nutrition vs. randomized controlled trials**

Coherence between lifestyle factors, including the composition of our diet, is quite obvious from an evolutionary point of view. After all, there was first an environment, and from this environment originated a genome that was adapted to that environment: it is the substrate (environment) that selects the organism, not vice versa. This is exactly what Darwin meant with ‘conditions of existence’, as the most important driving force in evolution. In other words, our only slowly changing genome is indissolubly linked to a certain environment and lifestyle. However, we have changed this environment since the agricultural revolution and continue to do so with still increasing pace. The resulting conflict does not generate acute toxicity, but acts as an assassin in the long term. Probably, the conflict does not exert much selection pressure either, because its associated mortality occurs mainly after reproductive age.

To solve the conflict, it is virtually impossible to study all of the introduced errors in our lifestyle (Table 1) in isolation, according to the reigning paradigm of EBM (307). EBM is widely confused with the results of RCTs and preferably the meta-analysis thereof (308, 309). This paradigm, originally designed for objective evaluation of medical treatments and drugs in particular, and named in nutrition research ‘Evidence Based Nutrition’ (EBN); is at present misused by food scientists and Health and Nutrition advisory boards. In contrast to drugs, this (expensive) RCT paradigm usually lends itself poorly for the study of single nutrients with meaningful outcomes (308). For each nutrient, we are dealing with poorly researched dose-response relationships, multiple mechanisms of action, small effects causing pathology in the long-term, numerous interactions, ethical limitations regarding the choice of intervention and control groups, and the inability to patent its outcomes (309). The RCT criteria are moreover inconsistently applied in the current development of nutritional recommendations. For example, there is no RCT-supported evidence for the saturated fat hypothesis (170), and also not for the trans fatty acids, while such an approach is considered mandatory for the adjustment of the vitamin D nutritional standards (310-312). Incidentally, there was also no RCT prior to the introduction of trans fatty acids showing that they could be consumed without adverse effects on the long term. However, there is an RCT on the effects of smoking cessation, which showed an equal mortality among the quitters (313, 314). The meta-analyses of RCTs studying the influence of LCP on brain development are negative (315-318). However,
recommendations for their addition to infant formulas have been issued (196), probably because nobody wants to take chances with the brains of our offspring. By applying EBM in a rigorous manner and by merely taking a view from the ‘precautionary principle’ (i.e. zero risk 5) this well meant concept has become a burden in the nutritional science, that calls for replacement by appropriate risk-cost-benefit analyses such as e.g. performed for vitamin D (319).

Our diet is composed of millions of substances that are part of a biological network. In fact, we eat ‘biological systems’ like a banana, a fish or a piece of meat. There is a connection between the various nutrients in these systems. In other words, there is a balance and an interaction that is part of a living organism. This balance can be found in the reconstruction of our Paleolithic diet, and various attempts for this reconstruction have already been made (28, 131, 320-322). Preliminary results of interventions with a Paleolithic diet are utterly positive (for a review see (323)). For example, in an indeed uncontrolled study with non-obese sedentary healthy subjects, an eucaloric Paleolithic diet resulted within 10 days in beneficial effects on three out of the four symptoms of the metabolic syndrome, i.e. blood pressure, dyslipidemia and glucose homeostasis. The fourth symptom, overweight/obesity, was deliberately not changed to prevent the attribution of any beneficial changes to weight loss (324).

Nurture, not nature

Less than 5% of our diseases can primarily be ascribed to heritable genetic factors (325, 326). ‘Genome wide association studies’ (GWAS) will not make this figure change; not even if the number of patients and controls are further increased. As it could have been predicted from evolution, these GWAS identify only a few genes that are associated with typically Western diseases. Moreover, the so far identified genes merely convey low risks. In one of these disappointing GWAS, where 14,000 patients with seven major typically Western diseases and 3,000 controls were studied, it was concluded that: ‘... for any given trait, there will be few (if any) large effects, a handful of modest effects and a substantial number of genes generating small or very small increases in disease risk’ (327). The differences in genetic susceptibility to environmental factors is widely confused with a primary genetic origin of Western disease. Environmental factors may mimic genetic heritability, especially when the exposure has become widespread, As clearly explained by Rose (328): “If everyone smoked 20 cigarettes a day, then clinical, case-control and cohort studies alike would lead us to conclude that lung cancer was a genetic disease; and in one sense that would be true, since if everyone is

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5 The precautionary principle is a moral and political principle stating that, if an intervention or policy may cause serious or irreversible damage to society or the environment, the burden of proof lies with the proponents of the intervention or the measure if there is no scientific consensus on the future damage. The precautionary principle is particularly applicable in health care and environment; in both cases we deal with complex systems in which interventions result in unpredictable effects (source: Wikipedia).
exposed to the necessary agent, then the distribution of cases is wholly determined by individual susceptibility". In other words: ‘disease susceptibility genes’ is a misnomer from an evolutionary point of view.

Most of the currently demonstrated polymorphisms associated with typically Western diseases already existed when homo sapiens emerged about 160,000 years ago in East-Africa. After all, the largest inter-individual genetic variation can be found between individuals belonging to a single population (93-95% of the total genetic variation), and only little genetic variation is on the account of differences between populations belonging to a single race (2%) and between the 5 races (3-5%) (329). The allele that, according to current knowledge, is linked with the highest penetrance of type 2 diabetes mellitus in the general population (with Western lifestyle!) conveys 46% higher relative risk (RR=1.46) (330). In contrast, a woman with a body mass index (BMI) of 35 kg/m² has a one hundred-fold higher risk (RR=100) of diabetes mellitus type 2 (331), which translates into a 9,900% higher relative risk. ‘Genetic’ diseases with relative risks below 1.5 have no practical value in Public Health. They are only important to our understanding of the etiology of the concerning disease and for drug development (326), which is part of Health Care.

Between 70 and 90% of the cases of type 2 diabetes mellitus, CVD and colon cancer can be prevented by paying more attention to nutrition, smoking, overweight and lack of physical activity (325). Hemminki et al. (326) stated that ‘if the Western population was to live in the same conditions as the populations of developing countries, the risk of cancer would decrease by 90%, provided that viral infections and mycotoxin exposures could be avoided’. The popular counter argument that people in developing countries have (on average!) shorter life spans is not valid. The reason that we (on average!) live longer in Western societies, is mainly due to the strong reduction of infectious diseases (particularly in childhood), famine and violence (332, 333), and also in part on the account of Health Care. However, together with our increasing life expectancy, there is a decrease in the number of years without chronic disease (334).

**Conclusions**

It has become clear that most, if not all, typically Western chronic illnesses find their primary cause in an unhealthy lifestyle and that systemic low grade inflammation is a common denominator. From an evolutionary point of view, the current conflict between environment and our Paleolithic genome traces back to our brain growth and the ensuing intimate relationship between inflammation and metabolism. The present disbalance between inflammatory and anti-inflammatory stimuli does not originate from a single cause and can consequently also not be solved by a single ‘magic bullet’. Resolution of the conflict between
environment and our ancient genome might be the only effective manner to arrive at ‘healthy aging’ and to achieve this objective we might have to return to the lifestyle of the Paleolithic era according to the culture of the 21st century (16, 322).
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**Chronic inflammatory diseases are stimulated by current lifestyle: how diet, stress levels and medication prevent our body from recovering**

Margarethe M Bosma-den Boer
* Corresponding address
Email: mirjam@praktijkoberon.nl

Marie-Louise van Wetten
Email: kvwetten@gmail.com

Leo Pruimboom
Email: udgleo@yahoo.es

1 University of Gerona, Gerona, Spain
Abstract
Serhan and colleagues introduced the term “Resoleomics” in 1996 as the process of inflammation resolution. The major discovery of Serhan’s work is that onset to conclusion of an inflammation is a controlled process of the immune system (IS) and not simply the consequence of an extinguished or “exhausted” immune reaction. Resoleomics can be considered as the evolutionary mechanism of restoring homeostatic balances after injury, inflammation and infection. Under normal circumstances, Resoleomics should be able to conclude inflammatory responses. Considering the modern pandemic increase of chronic medical and psychiatric illnesses involving chronic inflammation, it has become apparent that Resoleomics is not fulfilling its potential resolving capacity. We suggest that recent drastic changes in lifestyle, including diet and psycho-emotional stress, are responsible for inflammation and for disturbances in Resoleomics. In addition, current interventions, like chronic use of anti-inflammatory medication, suppress Resoleomics. These new lifestyle factors, including the use of medication, should be considered health hazards, as they are capable of long-term or chronic activation of the central stress axes. The IS is designed to produce solutions for fast, intensive hazards, not to cope with long-term, chronic stimulation. The never-ending stress factors of recent lifestyle changes have pushed the IS and the central stress system into a constant state of activity, leading to chronically unresolved inflammation and increased vulnerability for chronic disease. Our hypothesis is that modern diet, increased psycho-emotional stress and chronic use of anti-inflammatory medication disrupt the natural process of inflammation resolution ie Resoleomics.

Keywords
Chronic inflammation, Central stress system, Nutrition, Resoleomics, Sympathetic-adrenal-medulla axis, Hypothalamus-pituitary-adrenal axis, Anti-inflammatory medication, Insulin resistance, Polyunsaturated fatty acids, Glycemic index
Introduction

The number of people suffering from chronic diseases such as cardiovascular diseases (CVD), diabetes, respiratory diseases, mental disorders, autoimmune diseases (AID) and cancers has increased dramatically over the last three decades. The increasing rates of these chronic systemic illnesses suggest that inflammation [1,2], caused by excessive and inappropriate innate immune system (IIS) activity, is unable to respond appropriately to danger signals that are new in the context of evolution. This leads to unresolved or chronic inflammatory activation in the body.

Inflammation is designed to limit invasions and damage after injury, a process which has been essential for the survival of Homo sapiens in the absence of medication such as antibiotics. Recently, it has been discovered that onset to conclusion of an inflammation is a self-limiting and controlled process of the immune system (IS). This process of inflammation resolution is defined by Serhan as Resoleomics [3], a term which will be used throughout this article.

Our genes and physiology, which are still almost identical to those of our hunter-gatherer ancestors of 100,000 years ago, preserve core regulation and recovery processes [4,5]. Nowadays our genes operate in an environment which is completely different to the one for which they were designed.

Modern man is exposed to an environment which has changed enormously since the time of the industrial revolution. In recent decades there has been a tremendous acceleration in innovations which have changed our lives completely. As a consequence, more than 75% of humans do not meet the minimum requirement of the estimated necessary daily physical activity [6], 72% of modern food types is new in human evolution [7], psycho-emotional stress has increased and man is exposed to an overwhelming amount of information on a daily basis. All these factors combine to produce an environment full of modern danger signals which continuously activate the IIS and central stress axes. The question is whether the IIS and its natural inflammatory response, Resoleomics, can still function optimally in this modern, fast-changing environment, considering that the IIS is designed to produce short, intensive reactions to acute external danger [8,9]. It would seem that in the bodies of people who have adopted a Western lifestyle the inflammatory response is not concluded because of an initial excessive or subnormal onset of the response [10].

This article postulates how triggers from chronic altered diet and psycho-emotional stress negatively influence Resoleomics, thereby increasing susceptibility to the development of chronic, low-grade, inflammation-based diseases due to the constant activation of both the central stress axes and the IIS. In addition, an attempt is made to demonstrate the ways in which the use of anti-inflammatory medication could influence Resoleomics.
**Resoleomics, a self-limiting process of inflammation**

Serhan and his colleagues [3] introduced the term Resoleomics to describe a self-limiting process of inflammation, executed and controlled by the innate immune system (IIS) and regulated by the sympathetic nervous system (SNS) and the hypothalamus-pituitary-adrenal (HPA) axis. This process controls inflammation using metabolites produced from arachidonic acid (AA), eicosapentaenoic acid (EPA) and docosahexenoic acid (DHA). Resoleomics operates locally when polymorphonuclear neutrophils (PMNs) are attracted by increased pro-inflammatory cytokine and eicosanoids production during microbial invasion, wound healing or chemical injury. The function is to limit the inflammation response. The central control system of the inflammatory reaction is very complex. Local and central processes influence each other and both are responsible for an optimal resolving response (Figure 1). The local process can be divided into three phases [11] (Figure 2):

**Figure 1** Start and finish of a physiological inflammatory reaction in wound healing and situations of microbial challenge.

*Cellular damage and leakage of alarmins attract neutrophils to the damaged area (PMN’s). Sympathetic afferents activate the locus coeruleus (central nucleus of the sympathetic nervous system, SNS) and Noradrenaline (Norepinephrine, NE) is released. The released NE activates the adrenal medulla inducing the production of systemic catecholamins that supports the activation of the PMN. Damaged blood vessels are a source of an omega 3 rich edema (EPA and DHA). DHA and EPA inhibit LOX-5 directly and through conversion into resolvins and protectins. Both PGE2 and PGD2, produced by the breakdown of AA by COX-2 activity, will now override the strong chemotaxic effect of LTB4. The combined action of protectins, resolvins and lipoxins produced out of AA will put a hold on the pro-inflammatory activity of PMN’s, which is supported by the increased production of systemic cortisol. Cortisol further activates macrophages (M-Ph) to phagocytose me...*
issue debris and quiet PMN by releasing substances such as LXA4, resolvin E1 (RvE1), prostanoid D1 (PD1), fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF) and epithelial growth factor (EGF) at the same time. Further edema leakage will be stopped, whereas angiogenesis and production of connective tissue will take place, finishing the inflammatory reaction and starting the production of new tissue.

Figure 2 Inflammation is a controlled process with an initiation, resolution and termination phase.

After microbial invasion, lesion or chemical injury, the initiation phase starts with the production of pro-inflammatory mediators like LTB4 and PG2. These mediators increase inflammation until the Eicosanoid Switch, the end of the initiation phase, takes place. This occurs when the level of PGE2 plus PGD2 is equal to the LTB4 level. The resolution phase is entered, triggering the generation of anti-inflammatory mediators like LK, resolvins, protectins, maresins, PGD2 and PGF2a. When the total level of anti-inflammatory mediators exceeds the level of LTB4 the Stop Signal takes place. This is the last phase, the inflammation will be terminated by clearing the affected area [11]. The stress hormones produced by the systemic stress axes have a direct effect on the inflammation phases. A microbial invasion, lesion or injury sends off an alarm in the body, setting off the systemic stress system which produces NE as response and tunes the system to insulin and cortisol resistance [12]. The Eicosanoids Switch to resolution can only take place when NE is equal to the level of cortisol plus insulin and when cortisol sensitivity is recovered. The Stop Signal requires a low level of NE and normalized cortisol sensitivity. The termination phase is entered when the stress axes are switched off.
1. Initiation phase
2. Resolution phase
3. Termination phase

Initiation phase
Pro-inflammatory eicosanoids, like leukotrienes B4 (LTB4) and prostaglandins (PGs) initiate the inflammatory response. PMNs generate LTB4 and PGE2 from precursor AA with the use of lipooxygenase-5 (LOX-5) and cyclo-oxygenase 2 (COX-2). Both eicosanoids enhance inflammation, LTB4 being the strongest chemotoxic compound of cytotoxic neutrophils. PGE2 and/or PGD2, although initially pro-inflammatory, determine the switch to the next phase, the resolution of the inflammation.

Resolution phase
This phase starts with the Eicosanoid Switch to resolution. When the PGE2 and/or PGD2 level is equal to the level of LTB4, the PMNs activate the switch from pro-inflammatory to anti-inflammatory eicosanoids production by limiting the production of LOX-5. This switch is responsible for the production of anti-inflammatory lipoxins (LXs) from AA through activation of lipooxygenase –12 (LOX-12), lipooxygenase-15 (LOX-15) and acetylated COX-2 [13,14]. This last mechanism has been found to be responsible for the production of more stable aspirin-triggered LXs (ATLs) with a longer half-value period [15]. Other resolving metabolites that support LXs are resolvins, (neuro)protectins and maresins produced from respectively EPA and DHA [11,16]. A second substantial increase of COX-2 activity will produce anti-inflammatory PGs (PGD2 and PGF2a) during this phase [17].

Termination phase
This phase starts when the Stop Signal takes place. This happens when sufficient anti-inflammatory mediators such as LXs are available to stop the pro-inflammatory process [13,14]. LXs are capable of inhibiting both PMN infiltration and the activity of cytotoxic cells of the ISS, inducing phagocytosis to clear debris by non-cytotoxic macrophages and attenuating an accumulation of the pro-inflammatory transcription factors, ie nuclear factor-kappaB (NF-kB) and activator protein 1 (AP-1) [18,19].
Central stress axes and Resoleomics

This section deals solely with the effect of the sympathetic, parasympathetic and the HPA axis on Resoleomics. The systemic stress system is closely linked to the IIS via the stress axes of our body. Anything that can activate the sympathetic-adrenal-medulla (SAM) and HPA axes will have its effect on the IIS [20] and therefore on Resoleomics. Seen in reverse, it is precisely the IIS that can trigger stress axes, inducing a systemic stress reaction in the body [21]. In the SNS, which initially activates the IIS, inhibition of the IIS is provided by the strong anti-inflammatory neurotransmitter acetylcholine (ACh), produced by the parasympathetic nervous system [22].

The systemic stress reaction follows a two-wave pattern. Activation of the SAM axis is considered the first wave, giving rise to the excretion of brain norepinephrine (NE) by the Locus Coeruleus (LC). The descending pathway activates sympathetic motor neurons in the medulla oblongata, which stimulate the adrenal glands (through sympathetic efferent nerves). The adrenal gland will now excrete catecholamines, which activate and induce proliferation of ISS cells. NF-κB increases pro-inflammatory cytokines production, such as interleukin 1-beta (IL1-β), interleukin 6 (IL-6) and tumor necrosis factor (TNF). Both the IIS and Th1 of the adaptive IS contain receptors sensitive to catecholamines. Cerebral catecholamines affect the activity of spleen, thymus, bone marrow and lymphoid nodes [23]. NE has been shown to activate the IIS at the onset of inflammation, while long-term activation of the SNS induces IIS inhibition [24].

The second wave of the systemic stress reaction corresponds with the activation of the HPA axis, with glucocorticoids (GCs) as end product. Cortisol is capable of inhibiting the IIS through the upward regulation of inhibiting factor kappa B (IκB), while informing the immunological cortex through the migration of different immune cells to the brain [25,26]. Cortisol, the regulator of the IIS response, can guide the inflammation into resolution phase. Termination is instigated when cortisol “overrules” the NE effect on NF-κB signalling through genetic influence and reduction of transcription of the NF-κB sensitive pro-inflammatory gene, resulting in the finalization of the inflammatory response (Figure 2).

This “termination” effect of cortisol is normally supported by a compensatory anti-inflammatory response through activation of the vagal anti-inflammatory loop [27]. The resulting production of ACh inhibits the IS through the alpha-7-nicotin-Acetylcholinergic Receptor (α7nAChR) [28] (Figure 1).

The SNS (NE) increases the initial pro-inflammatory immune response in the initiation phase, whereas delayed cortisol response, induced by the HPA axis, inhibits the pro-inflammatory
response [29]. Integrity of the SAM axis with its NE response/ reaction is necessary for an adequate initial inflammatory response [30]. At the beginning of the initiation phase, there is resistance to both cortisol and insulin in order to allow for the activation of the IIS [12]. At the end of this phase, cortisol sensitivity and insulin sensitivity should be recovered to facilitate the Eicosanoid Switch to the resolution phase.

Chronic stress exposure reduces the capacity to mount an acute stress response [31], resulting in an inadequate pro-inflammatory response. Chronic (psycho-emotional) stress situations can be responsible for the continuous production of catecholamines by the SAM axis. People suffering from "perpetual stress", for example the parents of a child with cancer, showed chronic, increased levels of circulating pro-inflammatory cytokines [26]. This situation requires a high level of energy expenditure. The metabolic rate is increased to provide extra energy for the brain (arousal of all senses), the heart muscle and the locomotive system. The existing cells from the IIS are activated and will proliferate (relatively low energy expenditure), whereas proliferation of new immune cells (much more costly energy expenditure) will be blocked. Further consequences of chronic SAM activity are narrowing of the cell spectrum of the IIS and complete loss of activity of the Th1 section of the adaptive IS, leading to an insufficient capability to fight viruses, (pre)neoplastic cells and intracellularly presented pathogens [31].

An inflammatory response leading to solution depends on the sensitivity of glucocorticoid receptors (GR) and catecholamine receptors of the IIS [32]. Factors such as stress endured early in life, trauma and polymorphisms are possible risk factors for loss of GR and catecholamine sensitivity [33-35].

Suboptimal inflammatory response as a consequence of chronic stress prevents the Eicosanoid Switch from functioning, since the switch to the resolution phase requires recovered cortisol and insulin sensitivity. The initiation phase should have a maximum duration of 8 to 12 hrs. PMN number and activation levels should reach their maximum during this phase; longer duration caused by chronic stress could produce secondary damage to neighbouring tissues due to the strong cytotoxic effects of activated PMNs [11]. Supramaximal activation of PMNs could sensitize the adapted IS if contact time between self-antigens and the IS is significantly increased [11,29].

The crosstalk between the IS and stress axes is further evidenced by the fact that acute production of high levels of catecholamines activate the IIS strongly [23], whereas eicosanoids produced from AA induce the production of local and systemic catecholamines [36]. Long-term activation may lead to catecholamine resistance and lack of eicosanoid production. This situation, combined with the aforementioned possibility of resistance to
insulin and cortisol, provokes a suboptimal inflammatory response and consequently the perpetuation and development of low-grade inflammation [26,37].

**Nutritional factors and Resoleomics**

Several dietary factors influence the activity of the IIS and the function of a wide range of hormones, including cortisol, insulin and catecholamines. The dramatic changes in dietary composition since the agricultural revolution (some 10,000 years ago) and, to a greater extent, since the industrial revolution (some 200 years ago) have turned the intake of food into a common daily danger and therefore a cause of continuous systemic stress. Some of these changes include an increase in the omega 6/omega 3 fatty acid ratio, a high intake of saturated fatty acids (SFA) and refined carbohydrates, the introduction of industrially produced trans fatty acids, a lower intake of vitamins D and K, imbalanced intake of antioxidants, high intake of anti-nutrients (eg lectines, saponins) and an altered intake of dietary fibre [38].

The following section will discuss the impact of the changed ratio of polyunsaturated fatty acids (PUFAs) and the intake of food with a high glycemic load on Resoleomics. The pro-inflammatory effects of anti-nutrients present in cereals [39], potatoes [40], legumes [41], and tomato have previously been extensively reviewed [7].

**Role of PUFAs in inflammation**

The intake ratio of \( \alpha \)-linoleic acid (LA) (omega 6), \( \alpha \)-linolenic acid (ALA) (omega 3), docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) in the Western diet has changed dramatically compared to the estimated intake ratio of hunter-gatherer diets from 2–3:1 to 10–20:1 in the contemporary diet [42,43]. All of these PUFAs are essential for normal Resoleomics response, as they function as precursors for the special small mediators responsible for the instigation and conclusion of the inflammatory response. One of the toxic changes in fatty acid composition of food corresponds to the increased intake of LA since the production of vegetable oils in 1913. Increased LA levels affect the inflammation process in three ways (Figure 3):
Summary of the effects of high LA intake on Resoleomics

1. Increase of the omega 6 / omega 3 fatty acid ratio
2. Altered AA level
3. Increases of inflammatory compounds, leukotoxins (LK) production

Increased omega 6 / omega 3 fatty acid ratio

The inflammatory effect of a high omega 6/omega 3 fatty acid ratio during inflammation has been demonstrated in recent human studies [44,45], in vitro studies [46,47] and animal studies [48,49]. The higher LA levels in phospholipids in plasma and cell membranes seem to be a major factor responsible for incomplete Resoleomics reactions. Higher intake of omega 3 fatty acids in the form of DHA and EPA regulate the production of pro-inflammatory cytokines and decrease LA levels in phospholipids in plasma and cell membranes [46,48]. The conversion of LA and ALA into respectively AA, DHA and EPA depend on the same enzymes in the desaturase and elongase cascade, with δ-6-desaturase as the rate-limiting enzyme (Figure 4) [50].
Figure 4 Synthesis of unsaturated fatty acids in mammals by Desaturase and Elongase

Human trials investigating the effects of omega 3 dietary supplements showed significant improvements of symptoms in patients suffering from diseases such as RA, inflammatory bowel disease, asthma, psoriasis, breast cancer and CVD. However, full remission of symptoms was not achieved [43,51]. Our conclusion is that an increased intake of omega 3 alone is not enough to restore Resoleomics; the intake of LA must be decreased as well.

LA effect on AA level

Higher AA levels in plasma result in more adequate inflammatory reactions, since AA is a precursor of pro- and anti-inflammatory substances within the self-limiting inflammatory process [52]. LA is the precursor for AA in the desaturase/elongase conversion (Figure 4). Theoretically, LA could be the source of a sufficient level of endogenous AA. However, higher intake of LA does not deliver increased levels of AA in comparison to low intake [53,54]. To achieve the required AA level, AA should be present in the regular diet [45]. The combined situation of AA deficiency together with a reduced intake of omega 3 fatty acids such as DHA and EPA (necessary for the flip flop reaction of LOX-5 and the Eicosanoid Switch [3]), enable a perpetuation of the pro-inflammatory initiation phase and therefore of chronic inflammation.
Increased production of leukotoxin

The third harmful effect of high LA intake is the possible production of so-called leukotoxins (LK). High LA levels are metabolized by CYP2C9 in the liver into biologically active oxidation products known as LK and leukotoxin diol (LTD). These metabolites promote oxidative stress responses and the activation of NFkB and AP-1, increasing the systemic release of pro-inflammatory cytokines [55]. LK and LTD are toxic for T cells, and can kill these cells with pathways resembling necrosis and programmed cell death [56].

Role of high glycemic food in Inflammation

An abundant intake of high glycemic food appears to be related to an increased susceptibility to the development of chronic inflammation, as has been demonstrated by several research groups [57-59]. The consequences of a high carbohydrate diet are complex and multiple. The pathways leading to disturbances of normal inflammation are:

1. High glycemic food intake increases inflammation markers
2. High glycemic food intake causes hyperglycemia and hyperinsulinemia leading to disturbed balances in insulin growth factor-1 (IGF-1) and androgens
3. Chronic intake of high glycemic food causes hypoglycemia, which triggers central stress axes

High Glycemic food increases inflammation markers

Various clinical trials have shown that an abundant intake of high glycemic food increases inflammatory markers and markers of metabolic syndrome such as postprandial NFkB in mononuclear cells [57], high sensitive-C-Reactive Protein (hs-CRP)[58], interleukin (IL)-6, IL-7, IL-18 [60], levels of free radicals [59], cholesterol, triglycerides [61] and even blood pressure [62]. Changes incurred by following a low glycemic diet include improved insulin sensitivity, lower blood pressure and total cholesterol, which are all key markers of the metabolic syndrome [58,60,61]. The high glucose-induced inflammatory response is accompanied by hyperinsulinemia and insulin resistance, characteristic for people suffering from obesity [57,59]. Increased hsCRP values, hyperinsulinemia and insulin resistance are strongly related to CVD risk [60]. Glycemic index (GI) and glycemic load (GL) have therefore been proposed as biomarkers and predictors for (chronic) inflammation [63].

Hyperglycemia and hyperinsulinemia

Cordain demonstrated that high glycemic food is a potential risk factor for inflammation through disturbed signalling of mechanisms as a result of hyperglycemia and hyperinsulinemia [64] (Figure 5b). Long exposure to high glucose levels in blood, which leads to a slow recovery of the homeostasis, makes tissues vulnerable to disease [65]. High plasma...
insulin can increase the production of IGF-1 and androgens. Both hormones are related to disorders such as polycystic ovarian syndrome (PCOS) [66], epithelial cell cancer (breast, prostate, colon) [67,68], acne [69], androgenic alopecia [70], and acanthosis nigricans [71]. Several pathways in this respect have been previously described in medical literature, but these go beyond the scope of this article.

Figure 5  High glycemic food intake could cause inflammation and diseases as a result of hyperinsulinemia. The pathways in the shaded area have been extensively described by Cordain [64] (part B). Part A: The consequential reactive hyperglycemia is another deleterious pathway. Hyperglycemia is a danger signal, which activates the systemic stress system. Chronic activation will suppress the IIS, resulting in low grade inflammation and an increased vulnerability for excessive inflammation.

Hypoglycemia triggers the systemic stress system
As previously mentioned, intake of a high glycemic diet can cause hyperglycemia and hyperinsulinemia. Hyperglycemia will push abundant glucose via insulin into muscle and adipocytes at the instigation of the inflammatory process. However, continuous intake of high glycemic food results in reactive hypoglycemia, ie an energy-deficient situation which
threatens the homeostasis of the body. As a consequence, the brain will maintain its own energy supply aimed at the survival of the organism (the selfish brain) [25]. To ensure sufficient energy supply, the brain activates its systemic stress system to induce gluconeogenesis (Figure 5a). Excreted catecholamines and cortisol will mobilize extra energy, which is allocated with priority to the brain and to the activated IS, at the expense of other body tissues [72].

On the basis of the above information and other referenced data, it seems plausible to state that aspects of the Western diet, of the modern industrialised environment and of their resultant lifestyles form a chronic danger to the body, triggering both the central stress axes and the IIS into a state of chronic activity. This state seems to be a direct cause of the development of low-grade inflammation and consequently of chronic inflammatory diseases (Figure 5a).

Impact of current medication on Resoleomics

The role of the IIS is to limit the damage of inflammation in acute situations. Anti-inflammatory medication can be used to dampen the immune response. Nowadays, as a result of lifestyle changes, man is exposed to chronic inflammation and consequently to the chronic use of anti-inflammatory medication, much of which in fact suppresses Resoleomics. Current medication used to treat chronic inflammatory diseases does suppress the symptoms of inflammation, but complete remission of the disease is seldom realized [73]. Resoleomics is hindered and complete resolution of the inflammation does not take place. Modern chronic inflammatory diseases are treated by several groups of medication. In this article we focus on rheumatoid arthritis (RA) medication as an example. Four groups of anti-inflammatory RA medication are taken into account: the prostaglandin inhibitors [Nonsteroidal anti-inflammatory drugs (NSAIDs: Aspirin (ASA) and COX-inhibitors), the Glucocorticoids (GCs), the Disease Modifying Drugs (DMARDS: Methotrexate (MTX) and Sulfasalazine (SSZ)) and the cytokine blockers (Biological agents: anti TNF-α and IL-1 blockers]. The mechanisms of action and possible effects on the IIS and Resoleomics are summarized from literature (see Table 1). Most current therapies target the IIS in an attempt to inhibit the production of pro-inflammatory chemical mediators (Table 1). However, an equally important target is the active induction of pro-resolution programs by stromal cells such as fibroblasts within the inflamed tissues [74]. Inhibition of MIF [75] and production of NO [76] are not addressed in this article.
<table>
<thead>
<tr>
<th>Medication</th>
<th>Mechanism of action</th>
<th>Current RA treatment effects on immune system cells</th>
<th>Predicted effects on Resoleomics Phase 1: initiation, Phase 2: resolution, Phase 3: termination</th>
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</thead>
<tbody>
<tr>
<td>NSAIDs: Aspirin (ASA) [11,14,32,77-83]</td>
<td>COX-1 inhibition, COX-2 inhibition; PGE2 ↓; ATLs (15-epi-LX) ↑; Activation ALX/FPR2 receptor; PLA2 ↓; PGE &amp; LT ↓; PMN infiltration ↓, PGEs ↓, chemokines ↓; Leucocyte accumulation ↓; Neutrophil recruitment ↓</td>
<td>Negative: PG &lt; LT levels: phase 1 ↑; switch from phase 1 to phase 2 ↓; switch from phase 2 to phase 3 ↓; Cortisol resistance: switch from phase 1 to phase 2 ↓; or no switch; Lipoxins ↓: switch from phase 2 to phase 3 ↓</td>
<td></td>
</tr>
<tr>
<td>NSAIDs: COX inhibitors [84,85]</td>
<td>COX-2 inhibition &gt; COX-1 inhibition; PGE2 ↓, LTB4 ↑, PGF2α ↓, PGD2 ↓</td>
<td>Negative: PGE, LT ↓: switch from phase 1 to phase 2 ↓; Lipoxins: switch from phase 2 to phase 3 ↓</td>
<td></td>
</tr>
<tr>
<td>Glucocorticosteroids [32,80,86,87]</td>
<td>Transcription of IKB ↑: NFkB ↓; Transcription by GCR &amp; CREB-binding protein (CBP) ↓; PLA2 ↓: free AA, PGE &amp; LT ↓; Annexin-1 ↑; Activation of the ALX/FPR2 receptor; PMN infiltration ↑, PGEs ↑, chemokines ↑; Leucocyte accumulation ↑; Neutrophil recruitment ↑</td>
<td>Negative: PGE, LT ↓: switch from phase 1 to phase 2 ↓; Lipoxins: switch from phase 2 to phase 3 ↓</td>
<td></td>
</tr>
</tbody>
</table>
DMARDs:

- Methotrexate (MTX)

Folate analogs: 1. Folate-dependent enzymes ↓:
   - 1a. Thymidylate synthetase; 1b. AICAR transformylase; 1c. Dihydrofolate reductase; 2. Cytosol peroxide (ROS) ↑:

Ad 1a. Synthesis of DNA & RNA ↓; T-cell proliferation & protein & cytokine expression ↓; LT & IL-1 ↓;
Ad 1b. Adenosine ↑: NK-cell, monocytes & macrophages functioning ↓; Cytokine synthesis TNF-α, IL-1, IL-6 & IL-8 ↓;
Ad 1c. THF ↓: purine & pyrimidine ↓;
Ad 2. T-cell apoptosis ↑

Negative: cytokines ↓, T-cell activity ↓, LT ↓; switch from phase 1 to 2 ↓ or no switch

Biological agents: Anti-TNF-α [54,103-105]

TNF-α signalling to monocytes, PMN’s, T-cells, endothelial cells, synovial fibroblasts, adipocytes, etc.

Cytokine release ↓; MMP-2 & MMP-9 release ↓; PMN priming, apoptosis & oxidative burst ↓;
Monocyte activation, cytokine & PG release ↑; PMN ↑;
Cox-2 & PGE2 ↑; IL-1 & TNF-α ↑; Antibody plasma cells ↓;
Neutrophils, monocytes, macrophages, granulocytes activation ↓; Switch from phase 1 to 2 ↓

Ad 1a. Anti-TNF-α; Ad 1b. Adenosine receptor antagonists; Ad 1c. Dihydrofolate reductase inhibitors; Ad 2. T-cell apoptosis ↓

DMARDs:

- Sulphasalazine (SSZ)

SSZ: strong & potent inhibitor of NFkB activation; 5-ASA: PG ↓; sulpha-pyridine metabolites NFkB activation ↓: IL-2 activated T-cells ↓, TNF-α & IL-1 macrophages ↓; Antibody plasma cells ↓;
Neutrophils, monocytes, macrophages activation ↓, IκB ↓: NFkB translocation ↓ & transcription of cytokines, adhesion molecules, chemokines ↓: COX-2 & PG ↓

Negative: Immune cell activity ↓; switch from phase 1 to 2 ↓

Biological agents: Anti-TNF-α ↓; TNF-α expression ↓; PMN ↑; PMN activation, cytokine & PG release ↓; PMN priming & apoptosis; T-cell apoptosis, clonal regulation & T-cell receptor ↓;
Cox-2 & PGE2 ↓; IL-1 & TNF-α ↑; Antibody plasma cells ↓;
Neutrophils, monocytes, macrophages, granulocytes activation ↓; Switch from phase 1 to 2 ↓

Ad 1a. Anti-TNF-α; Ad 1b. Adenosine receptor antagonists; Ad 1c. Dihydrofolate reductase inhibitors; Ad 2. T-cell apoptosis ↓

DMARDs:
Biological agents: IL-1 blocker [54, 103-105]

- IL-1 signalling monocytes, B-cells, synovial fibroblasts, chondrocytes
- COX-2 induction ↓
- Synovial fibroblast cytokine, chemokine, MMP, iNOS & PG release ↓
- Monocytes cytokine, ROI & PG release ↓
- Osteoclast activity ↓
- Synovial fibroblast cytokine, chemokine, MMP, iNOS, aggrecanase ↑
- Endothelial cell adhesion molecule expression ↓
- Negative: Immune cell activity ↓

Switch from phase 1 to 2

↑ Blocker [54, 103-105]

- III-1 signalling monocytes, B-cells, synovial fibroblasts, chondrocytes
- IL-1 blocker, synovial fibroblasts, chondrocytes
Positive effect of ASA and GCs on Resoleomics

Medical intervention should stimulate the endogenous pathways of resolution and two drugs already known to possess these qualities are central to contemporary medicine: glucocorticoids (GCs) [77] and aspirin (ASA) [106,107]. It is apparent that ASA and GCs have a positive effect on Resoleomics, while other medications prolong the initiation phase, tempering and/or blocking the resolution and termination phase of Resoleomics in various ways (Table 1). The positive effect of ASA on Resoleomics can be ascribed to its ability to produce ASA-triggered lipoxins (ATLs) through acetylation (and not through an irreversible inhibition) of the COX-2 enzymes [78]. These ATLs show many pro-resolving properties, which are essential in the resolution and termination phase of the inflammation process [79,108]. Long-term intake of high doses of ASA blocks PGE2 production and initiates the resolution phase without affecting the biosynthesis of other pro-resolving mediators [108]. Low and high doses of ASA increase the production of lipoxin A4 (LXA4) and 15-epi-LXA4 in the rat brain, suggesting that ASA could protect against neuroinflammation [109]. However, because of its side effects, ASA is no longer the treatment of choice for RA. In high doses, inhibition of the COX-1 enzyme by ASA is responsible for damage to the stomach lining.

ASA and also GCs activate the ALX/FRP2 receptor, making them the ideal collaborator in the resolution process [77]. GCs-induced annexin-1 protein (ANXA1) [110,111] as well as ASA-induced ATLs act on the same ALX/FPR2 receptor and dampen PMN infiltration [77,80]. ANXA1 also inhibits the phospholipids A2 enzyme (PLA2). Reduced PLA2 activity appears to reduce AA release from the cell membrane [32,112], which possibly leads to decreased levels of both PGs and LTs and to the delay of resolution. Besides their anti-inflammatory effects, GCs have a positive influence on resolution by enhancing macrophage migration and phagocytosis [11,113].

Adverse effects of medication on Resoleomics

The use of anti-inflammatory medication without the capacity to induce (complete) resolution should be considered solution-toxic, i.e., hindering Resoleomics. NSAIDs are strong inhibitors of COX-2 and less of COX-1 enzymes [114]. Almost complete COX-2 inhibition decreases the PGs synthesis, and consequently leads to a higher production of LTs via LOX-5 in PMNs [115]. PGE2 and PgD2 decrease the activity of LOX-5, decreasing neutrophil activity and facilitating the end of the inflammatory phase and the instigation of resolution.

Immune-suppressors such as SSZ (and less powerful GCs) almost completely block NF-κB transcription, leading to insufficient cytokine production and suboptimal inflammation [86]. Again the resolution process will not be completed, with perpetuation of inflammation as the logical consequence.
Perhaps the most deleterious drugs, interfering negatively with resolution, are TNF-alpha inhibitors such as anti TNF-alpha and MTX. MTX inhibits the proliferation of the IIS cells, decreasing the production and accumulation of adenosine within the IS cells [88,116]. These effects lead to rapid anti-inflammatory effects and symptom release. However, because of its side effects and incomplete resolution, this medication is qualified as solution-toxic. This conclusion is supported by many patients who have discontinued this treatment [73].

Another group of possible solution-toxic drugs are biological agents with an inhibiting effect on TNF-alpha and IL-1. Biological agents together with DMARDS (Table 1) are strong anti-inflammatory compounds, decreasing the production of pro-inflammatory cytokines. The absence or insufficient activity of pro-inflammatory cytokines decreases cell communication and induction of COX-2 in activated neutrophils. This can lead to less production of resolution substances such as PgE2, PgD2 and lipoxins [54,103]. Furthermore, DMARDs and biological agents appear to reduce the functioning and number of IIS cells, causing suboptimal inflammation and possibly inflammation perpetuation [104].

**Discussion**

Long-term activity of the IIS results in low-grade inflammation and chronic disease. Over the past years, ideas regarding the treatment of inflammation have started to change as evidence accumulates which shows that, although the targeting of infiltrating immune cells can control the inflammatory response, it does not lead to its complete resolution and a return to homeostasis, which is essential for healthy tissue and good health in general.

Hotamisligil describes how low-grade, chronic inflammation ('meta-inflammation') induced by a nutritional and metabolic surplus, is accompanied by disturbed metabolic pathways and chronic metabolic disorders. He states that this inflammatory response differs from the classical inflammation response caused by injury [117]. However, others have shown that the classical response of the IIS dealing with injuries can be linked to activation of the central stress axes [26,28]. This article specifically discusses the relationship between the over-activated systemic stress system and the self-limited process of inflammation, known as Resoleomics, executed and controlled by the innate immune system (IIS).

Changes in lifestyle which are new to our evolutionary process should be considered a major trigger in causing chronic activation of the IS and consequently of the central stress axes and vice versa, thereby leading to chronic diseases such as cardiovascular diseases (CVD), diabetes, respiratory diseases, mental disorders, auto-immune diseases (AID) and cancers. This article evaluates two of the lifestyle changes which contribute to long-term activity of the ISS, namely, nutrition and continuous psycho-emotional stress. Other risk factors such as
Nutrition is an important factor in understanding the development of chronic inflammation. The current Western diet can disturb the resolution response in various ways (Figure 6). In the Ancestral human diet, foodstuffs with an increased risk of inflammation were virtually unknown, while nutrients able to activate the IIS are now abundant in our diet [38,120]. Cordain’s research has focused on relating these anti-nutrients in food (eg lectines, saponines) to the development of chronic inflammation and autoimmune diseases (AID) [7,39]. Fortunately, it seems that the human body possesses a strong capacity to recover from illness. If our genes are exposed to their ‘original’ environment by intake of an ancestral human diet, their function can recover rapidly. Research has shown that obese persons improve their blood markers after just 10 days following a paleolithic diet consisting of fish, lean meat, fruit, vegetables and nuts [121]. Similar results have been found in a study with aboriginals suffering from Diabetes II, who showed normalized blood markers after returning to their traditional lifestyle for seven weeks [122].

**Figure 6** Reflection of the working mechanism demonstrating how several nutritional factors could induce and inhibit inflammation
People suffering from chronic inflammatory disease demonstrate over-activated central stress axes, which then lead to catecholamines, cortisol and insulin resistance. McGowan et al [123] show the impact of childhood abuse on the epigenetic pattern of different genes including the gene for GR in the hippocampus. They found a decreased level of GR and an increased methylation pattern of the GR gene, giving rise to a situation of lower cortisol sensibility and altered HPA stress responses. This could make people more vulnerable to developing diseases. An altered sensitivity to cortisol has been linked to diseases such as rheumatoid arthritis (RA) [124], post-traumatic stress syndrome [125], chronic fatigue syndrome [126], inflammatory diseases and AID in general [127].

The key priority in the treatment of people with chronic inflammation is to induce the Eicosanoid Switch to the anti-inflammatory resolution phase. Long-lasting cortisol resistance and insulin resistance will definitely delay or block complete resolution. The combination of local factors (ie DHA deficiency, low levels of protectins) disturbing the process of complete resolution (ie Resoleomics) and the absence of adequate NE and cortisol signalling can be responsible for perpetual inflammation by delaying the resolution phase of the inflammatory response (Figure 7).

**Figure 7** Chronic over-activation of the systemic stress system as a result of external stressors plays a central role in the development of chronic inflammatory diseases.

*Current intervention with anti-inflammatory medication suppresses Resoleomics and the IIS and so enhance the over-activation of the systemic stress system*
Current anti-inflammatory medication used in RA treatment is aimed at the suppression of the IIS and its inflammatory response and thus hinders Resoleomics. In addition, these medication interventions do not solve underlying catecholamine, cortisol and insulin resistance, and consequently making it impossible to achieve full recovery of the chronic inflammation. This suggests that chronic use of anti-inflammatory medication in fact impedes the body from making a full recovery. Furthermore, the ongoing low-grade inflammation will continuously trigger the activity of the systemic stress system [28].

Health care should focus on early detection of silent, ongoing and low-grade inflammation in order to avoid the development of many chronic diseases. Further research is needed to validate a questionnaire which addresses early symptoms of chronic low-grade inflammation, ie avoidance of exercise, fatigue, emotional flatness, social isolation, decreased libido, hyper or hyposomnia, obsessive behaviour or sensitivity to addiction [6,128].

We have made an effort to demonstrate that the science of Resoleomics can help to find new ways to treat people suffering from diseases based on chronic inflammation. Since over-activated central stress axes directly delay Resoleomics, and thereby delay the resolution of inflammation, treatment should focus on restoring the central stress system to its default, healthy homeostasis. Dietary changes, psycho-emotional stress release and physical activity should always be included in treatment of all chronic inflammatory diseases.
Abbreviations

AA, Arachidonic acid; ACh, Acetylcholine; AID, Autoimmune diseases; ALA, α-linolenic acid; ALX/FPR2, Lipoxin A(4) receptor; ANXA 1, Annexin 1 protein; AP-1, Activator protein 1; ASA, Aspirin; ATLs, Stable aspirin-triggered lipoxin; COX, Cyclo-oxygenase; CRP, High sensitive-C-Reactive Protein; CVD, Cardiovascular diseases; DHA, Docosahexaenoic acid; DMARDs, Disease Modifying Drugs; EPA, Eicosapentaenoic acid; GI, Glycemic index; GL, Glycemic load; GCs, Glucocorticoids; HPA, Hypothalamus-pituitary-adrenal; IGF-1, Insulin growth factor-1; IS, Immune system; IIS, Innate immune system; IL, Interleukin; LA, α-linoleic acid; LC, Locus Coeruleus; LOX, Lipoxygenase; LK, Leukotoxins; LTs, Leukotrienes; LTD, Leukotxin diol; LXs, Lipoxins; MTX, Methotrexate; NE, Norepinephrine (ie noradrenaline); NF-kB, Nuclear factor-KappaB; NSAIDs, Nonsteroidal anti-inflammatory drugs; PCOS, Polycystic ovarian syndrome; PGs/ PGE2/ PGD2/ PGF2a, Prostaglandins/ prostaglandin E2, D2, F2a; PLA2, Phospholipase A2 enzyme; PMNs, Polymorphonuclear leukocytes; PUFAs, Polyunsaturated fatty acids; RA, Rheumatoid arthritis; SAM, Sympathetic-adrenal-medulla; SFA, Saturated fatty acids; SNS, Sympathetic nervous system; TNF, Tumour necrosis factor.

Competing interests

The author(s) declare that they have no competing interests.

Authors’ contributions

MMB executed an analysis and review of the relationship between chronic inflammatory pathways and the central stress systems, Resoleomics and nutrition. MMB also drafted the manuscript. MLvW reviewed the MOA of currently used anti-inflammatory medication and its effect on Resoleomics. LP played a central role in integrating the results of various stressors on chronic inflammation pathways and also acted as lead reviewer. All authors have approved the final manuscript.

Authors’ information

MMB and MLvW, MD treat patients with chronic diseases in a private practice. LP, a practising psychoneuroimmunologist and director of the master in CPNI at the University of Gerona, Spain, has developed valuable insights into the metabolic pathways of chronic diseases, which he has applied in the treatment of numerous patients.
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Stress induces endotoxemia and low grade inflammation by increasing barrier permeability

Karin de Punder¹,²,*, Leo Pruimboom¹, ³

1. Natura Foundation, Numansdorp, The Netherlands
2. Institute of Medical Psychology, Charité University Medicine, Berlin, Germany
3. University of Groningen, Groningen, Holland

Correspondence: Karin de Punder, Institute of Medical Psychology, Charité University Medicine, Berlin, Germany.
karin.de-punder@charite.de

Keywords: endotoxemia, endotoxin, inflammation, intestinal permeability, lipopolysaccharide, LPS, stress, tight junction
Abstract

Chronic non-communicable diseases (NCDs) are the leading causes of work absence, disability and mortality worldwide. Most of these diseases are associated with low-grade inflammation. Here we hypothesize that stresses (defined as homeostatic disturbances) can induce low-grade inflammation by increasing the availability of water, sodium and energy-rich substances to meet the increased metabolic demand induced by the stressor. One way of triggering low-grade inflammation is by increasing intestinal barrier permeability through activation of various components of the stress system. Although beneficial to meet the demands necessary during stress, increased intestinal barrier permeability also raises the possibility of the translocation of bacteria and their toxins across the intestinal lumen into the blood circulation. In combination with modern life-style factors, the increase in bacteria/bacterial toxin translocation arising from a more permeable intestinal wall causes a low-grade inflammatory state. We support this hypothesis with numerous studies finding associations with NCDs and markers of endotoxemia, suggesting that this process plays a pivotal and perhaps even a causal role in the development of low-grade inflammation and its related diseases.

Introduction

Inflammation is the response of the innate immune system triggered by stimuli like microbial pathogens and injury. Acute systemic inflammation such as in sepsis, trauma, burns, and surgery is characterized by a quick increase in plasma-levels (up to 100 fold) of pro-inflammatory cytokines and acute phase proteins, while in low-grade inflammation there is a sustained but only two to three fold increase in circulation pro-inflammatory mediators (1). Chronic low-grade inflammation is characteristic for many non-communicable diseases (NCD) including diabetes type II, cardiovascular disorders, autoimmune diseases, chronic fatigue syndrome, depression and neurodegenerative pathologies, but until now the exact mechanism behind the elevated levels of inflammatory mediators found in these conditions is not well understood (2-5).

Inflammation can be induced by the binding of pathogen-associated molecular patterns (PAMP) to toll-like receptors (TLR), which are expressed on different cells types including immune cells, adipocytes and endothelial cells. The most extensively studied PAMP is lipopolysaccharide (LPS) or endotoxin (the terms LPS and endotoxin will be used interchangeably throughout the rest of the article), a major cell wall component of Gram-negative bacteria, which is normally present in the human circulation in very low concentrations. It has been hypothesized that most of this circulating LPS is derived from the gut, since the gut-microbiota is the biggest source of Gram-negative bacteria-derived LPS. However, LPS found in the circulation could also be derived from Gram-negative bacteria
residing in the oral cavity, respiratory and genitourinary tracts, or can be food derived (6-8). Under certain circumstances there can be an increase of endotoxin translocation across the intestinal barrier, leading to mildly increased concentrations in blood circulation. This process has been associated with several NCDs, like depression (9), chronic fatigue syndrome (10), chronic heart failure (11), type 2 diabetes (12), autism (13), non-alcoholic fatty liver disease (NAFLD) (14) and inflammatory bowel disease (IBD) (15), diseases that are all linked to chronic systemic low-grade inflammation, indicating that endotoxemia could be an important contributor in the development of these conditions.

Here we hypothesize that stress-induction leads to a more permeable intestinal wall intended to facilitate an increase in the availability of water, sodium and energy-rich substances necessary to meet the increased metabolic demand induced by the stressor. Modern life-style factors, such as long-term psychosocial stress and components of our "Western" diet constantly challenge the stress-axis and further compromise intestinal barrier function, resulting in endotoxemia, low grade inflammation and its related diseases. We support our hypothesis by describing literature surrounding stress- and immune system activation processes and their relation to gut barrier function and explain how lifestyle choices impact all these systems. In addition we present a vast amount of literature describing associations with NCDs and markers of endotoxemia. Overall we conclude that stress-induced disrupted barrier function in parallel with elevated circulating endotoxin levels may underlie disease onset and progression and should be considered much more than just a risk factor for chronic disease; it could be a cause.

1. Bacterial toxins activate the immune system via TLR

LPS, the major cell wall component of Gram-negative bacteria, is characterized by its capacity to induce inflammation, fever, shock and death (1). Additionally in recent years, other cell wall components of Gram-negative and -positive bacteria, have been recognized to have endotoxic properties (16), but these will not be further addressed in the rest of the paper. Endotoxins are released from bacteria during infection or as a consequence of bacterial lysis. Although both whole bacteria and bacterial toxins can translocate transcellular or paracellular into the lymph, blood and mesenteric lymph nodes, it is still not precisely clear if the presence of endotoxin in the blood circulation (endotoxemia) also presents whole bacteria translocation across the intestinal wall (17).

Inflammation can be induced by the binding of LPS to TLR4. The lipid-A moiety of LPS interacts with the LPS-sensing machinery composed of TLR4, myeloid differential protein 2, CD14 and LPS-binding protein (LBP). LBP transports and delivers circulating aggregates of LPS to lipoproteins, resulting in hepatic clearance, or delivers LPS to CD14 (the membrane bound
or secreted, soluble form of this molecule), leading to TLR4 activation. TLR4 activation activates two transcription factors, activator protein (AP)-1 and nuclear factor κB (NF-κB) (18, 19), and stimulates the production of pro-inflammatory mediators such as prostaglandin 2 (PGE2) (20), tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, Interferon (IFN)-γ and the acute phase protein, C-reactive protein (CRP) (19). Simultaneously, an uncontrolled pro-inflammatory reaction is prevented by the induction of TLR4, NF-κB and AP-1 signaling inhibitors, which are probably involved in creating endotoxin tolerance (21). LPS tolerance is defined as a reduced responsiveness to a LPS challenge following a first encounter of endotoxin (22). It has been suggested that the dose of LPS exposure is important for determining the switch between LPS tolerance and priming. For example, in macrophages, high LPS concentrations induced a robust pro-inflammatory response in parallel with the activation of inhibitory feedback mechanisms. Lower concentrations of LPS, like those observed in NCDs, removed transcriptional suppressors on the promoters of pro-inflammatory genes and induced a mild but persistent expression of pro-inflammatory mediators (21, 23).

2. **Intestinal barrier function**

2.1. **The paracellular pathway is important for water, mineral and nutrient uptake**

The intestinal barrier allows for the regulated uptake of water, minerals and nutrients and protects the gut lumen from damage due to harmful substances. Components can cross the epithelial barrier by active transport and endocytosis (transcellular) or via the paracellular route. Because hydrophilic solutes are limited to cross lipid membranes of epithelial cells, the paracellular route is an important and major route for the transport of water, solutes and minerals across the intestinal barrier (24, 25). Active glucose, sodium and water uptake is mediated by the activity of sodium-dependent glucose co-transporters (SGLT) (26). The transcellular absorption of glucose and sodium and the resulting basolateral disposition of glucose and sodium by these transporters opens up the paracellular pathway structure and allowing the passive flow of water and small nutrients by creating an osmotic gradient (27).

Intestinal permeability is a measure of the barrier function of the gut and relates to the paracellular space surrounding the brush border surface of the enterocytes and the junctional complexes (28). The junctional complex, containing tight junctions, adherens junctions and desmosomes is an important regulator of the paracellular pathway and allows the passage of water, solutes and ions, but under normal conditions provides a barrier to larger molecules (28, 29). The claudin family of junctional transmembrane proteins have a substantial effect on paracellular permeability. While one group of sealing claudins makes the paracellular barrier less permeable, the other group of claudins is known to increase paracellular permeability by
the formation of pores that increase permeability for small solutes (30, 31). The expression of claudin proteins varies between tissues, explaining the variances in permeability of tight junctions among tissues (27). The paracellular pathway can be divided into the pore and non-pore pathway. The pore pathway is mainly controlled by the expression of claudins, while the non-pore pathway is more sensitive to cytoskeletal disruptions (30). Cytoskeletal rearrangements can be induced by phosphorylation of the regulatory myosin light chain (MLC), induced by MLC-kinase (MLCK). Phosphorylation of the MLC facilitates myosin binding to actin and therefore aids in cytoskeletal contractility. MLCK can be activated by cytokines such as TNF-α, causing increases in tight junction permeability by actomyosin contraction and reorganization of the tight junction (32, 33). In addition, SGLT1 activation and associated increases in tight junction permeability are also paralleled with phosphorylation of MLC, indicating that MLCK is an important mediator in tight junction and paracellular permeability regulation (25, 34) (Figure 1).

Increased intestinal permeability has been associated with autoimmune diseases, such as type 1 diabetes (35), rheumatoid arthritis, multiple sclerosis (36), and diseases related to chronic inflammation-like IBD (36, 37), asthma (38), chronic fatigue syndrome and depression (10, 39). It has been hypothesized that chronic intestinal hyper-permeability results in a pro-inflammatory phenotype induced by the enhanced paracellular translocation of microbial (and dietary) antigens across the gut barrier (40).

3. **Intestinal barrier function**

3.1. **Stress increases permeability of the intestinal barrier**

Stressful stimuli activate the sympathetic nervous system (SNS) and hypothalamic-pituitary-adrenal (HPA)-axis. Activation of both systems increases the availability of water, minerals and energy-rich substances in order to meet with the body’s metabolic demand (41, 42). The SNS responds instantly to physical and psychological stress by reallocating energy into different organs by neuronal regulation of heart rate, blood flow, release of catecholamines (adrenalin and noradrenalin) from the adrenal medulla (43) and stimulation of the renin-angiotensin-aldosterone system (44), involved in retention of water and sodium from the kidneys. In addition to the kidneys, water and sodium reabsorption can also be achieved at the level of the intestine. The intestinal wall is innervated by adrenergic sympathetic nerve fibers that upon stimulation increase water and sodium absorption (45, 46), which is paralleled by increases in intestinal permeability. The SNS-induced increase in permeability is likely mediated by β2-adrenergic receptors expressed on epithelial cells (47). Activation of the β2-adrenergic receptors stimulated SGLT1-mediated glucose absorption from the gut (48, 49) and the resulting basolateral disposition of glucose and sodium by these transporters opens up the
paracellular pathway (27) (Figure 1). Not surprisingly, blockage of the SNS by means of thoracic epidural anesthesia resulted in the blockage of the endotoxin-induced increase in intestinal permeability in rats (50).

Activation of the HPA-axis leads to the release of glucocorticoids that potentiate some of the actions of catecholamines. Essential to this response are the neurons in the paraventricular nucleus of the hypothalamus expressing corticotropin-releasing hormone (CRH) and other co-secretagogues, such as arginine vasopressin and oxytocin, both involved in the regulation of water homeostasis. Arginine vasopressin and CRH trigger the immediate release of adrenocorticotropic hormone (ACTH) from the anterior pituitary, which in turn induces the release of glucocorticoids and to some extend mineralocorticoids from the adrenal cortex, stimulating gluconeogenesis and increasing sodium and water retention respectively (51, 52). Intestinal permeability is regulated by several components of the HPA-axis.

In epithelial HT-29 monolayers, exposure to CRH resulted in an increased response to LPS as reflected by a decrease in transepithelial resistance and a significant increase in the expression of the pore forming protein, claudin 2 (27). Interestingly enough, these effects were mediated by an increase in TLR4 expression, an observation that could be repeated in mice treated with the water-avoid stressor (53). TLR4 activation resulted in the activation of the transcription factor NF-κB, which has specific binding sites in the claudin 2 gene promoter (54), indicating that in epithelial cells CRH affects both intestinal permeability and inflammatory pathways.

In rats, exposure to restricted stress or swimming stress increased intestinal permeability throughout the whole intestinal tract as measured by the fractional secretion of the urinary recovery of sucrose (reflecting gastric permeability), the lactulose-mannitol ratio (as a marker for small intestinal permeability) and sucralse (reflecting both small intestinal and colonic permeability) (55). Other experimental animal stress models such as thermal injury or early maternal deprivation induced the development of gastric ulcers, altered gastrointestinal motility and ion secretion, and increased intestinal permeability (reviewed by Caso et al. 2008 (56)). SGLT1 expression was markedly increased in the rat jejunum and ileum after 8 weeks of restraint stress. These findings were paralleled with an increase in intestinal lymphocytic infiltration and adrenal gland weight gain (26). The up-regulation of the SGLT1 is probably necessary to meet with the increased water, sodium and nutrient demand, induced by chronic stress (42).

The effect of acute stress on intestinal permeability was also investigated in humans (57). In healthy volunteers subjected to a public speech test, high cortisol-responders displayed
increased intestinal permeability as measured by the lactulose-mannitol ratio. Exogenous CRH administration also increased intestinal permeability, yet the CRH-induced hyperpermeability could be suppressed by the mast cell stabilizer disodium cromoglycate. Mast cell stabilization before the public speech test also did not alter intestinal permeability, however, it should be noted that in this experiment a control group was not included. Nevertheless, these results identify CRH as an important factor in the stress-induced alterations of the intestinal barrier function. These alterations seemed to be mediated by intestinal mast cells that upon activation secrete pro-inflammatory mediators like IFN-γ and TNF-α. A variety of pro-inflammatory cytokines increases epithelial and endothelial paracellular permeability by modulating the structure of the tight junction and by inducing cytoskeletal disruptions via activation of MLCK (32, 34, 58) (Figure 1). For example, IFN-γ increased epithelial permeability of T84 monolayers to large molecules (10 kDa). Interestingly, the IFN-γ-induced increase in permeability also up-regulated the passage of FITC-labeled-endotoxin by ten-fold (59).

4. **Neuroendocrine-immune interactions**

The complex neuroendocrine-immune interactions are evidenced by the fact that emotional stressors influence the immune response and that pure immunological stimuli impact on cognitive performance (60). Inflammatory mediators activate the HPA-axis with the purpose to provoke disease behavior and redirect energy-rich nutrients towards the immune system (61). Cytokines have been shown to increase nutrient availability to meet with the inflammation-dependent increased metabolic demand. For example, the cytokine Il-1α increased whole body glucose metabolism on a central level (62) and cytokines like Il-6, TNF-α, Il-1 and IFN independently evoke a HPA-axis response (63-65). Immune mediators can communicate with the brain via several pathways. By stimulating afferent sensory nerve fibers, entering the brain via the circumventricular organs or by binding to cerebral blood vessel endothelium immune mediators effectively redirect energy-rich substrates towards the immune system (41, 42).

Besides inflammatory cytokines, prostaglandins synthesized via the cyclooxygenase system play a central role in inflammation and HPA-axis activation. Zimomra et al. (65) demonstrated that in rats the initial activation of the HPA-axis by LPS is mediated by prostaglandins, like PgE2, while inflammatory cytokines maintain corticosterone levels at later time-points. In this study it was suggested that prostaglandins stimulated corticosterone release in a direct manner, since the peak in circulating corticosterone levels was observed long before the peak in circulating ACTH. This idea was confirmed by a study in rodents, showing that PgE2 directly stimulated the release of glucocorticoids from the adrenal gland (66). In human adrenal cells expressing TLR2 and TLR4, LPS stimulation resulted in the release of cortisol. This effect was mediated by PgE2, since inhibition of cyclooxygenase-2 attenuated cortisol
release (67).
As indicated, TLR4 activation stimulates the release of PgE2 by immune cells, adipocytes, endothelial, epithelial and probably also adrenal cells (68), inducing the peripheral release of glucocorticoids from the adrenal gland (66). PgE2 also activates glucocorticoid production through activation of the HPA-axis at the level of the hypothalamus and the pituitary (69). Macrophages, homing in blood vessels in the cranium, are directly activated by danger signals such as LPS. Activation of these special macrophages induces the production of PgE2 which directly stimulates the paraventricular nucleus of the hypothalamus, leading to higher production of glucocorticoids which should probably protect against possible inflammation of the brain (69).

5. **Acute stress increases pro-inflammatory pathways by increasing intestinal permeability**

Acute stress modulates the immune response and changes immune cell distribution. These neuroendocrine effects on the immune system are mediated by stress-hormones released from the adrenal gland, by direct innervation of sympathetic nerve fibers into lymphoid organs and by stress hormone receptors expressed on immune cells, like glucocorticoid receptors (GR) and α- and β-adrenergic receptors (70-72). It has been suggested that by mobilizing immune cells, the stress response, also known as the “fight-flight reaction”, prepares the immune system for oncoming challenges (70).

In addition, acute stress increases circulating pro-inflammatory mediators (73-75). In subjects exposed to acute stress, NF-κB was up-regulated in peripheral blood mononuclear cells in parallel with elevated levels of circulating catecholamines and glucocorticoids (76). Until now it is not completely understood what causes this pro-inflammatory response. Glucocorticoids mostly have an inhibitory effect on inflammatory pathways and catecholamines a rather modulating than activating influence on the immune system (71, 72, 77), however, it has been shown that activation of the β-adrenergic receptor by noradrenalin (but not adrenalin) increased NF-κB binding to DNA in monocytes in vitro (76). A recent study in rodents showed that acute stress-induced neuro-inflammation could be prevented by a pre-stress treatment with antibiotics or an inhibitor of MLCK. In addition, these treatments prevented stress-induced hyper-permeability and endotoxemia, indicating that it is not the stress-factor itself producing a pro-inflammatory response of the immune system, but the fact that stress increases barrier permeability and the translocation of endotoxin into the circulation. Pre-stress probiotic treatment with *Lactobacillus farciminis* had similar effects, which could be explained by its ability to enhance intestinal barrier function (78). In agreement with these results, it could be hypothesized that the (short lasting) pro-inflammatory activity in humans...
observed during acute stress is initiated by a stress-induced increase in intestinal permeability, mediated by the SNS and components of the HPA-axis, and resulting in higher levels of translocating endotoxin interacting with TLR on immune cells, adipocytes and epithelial cells. A schematic overview of the complex neuroendocrine-immune interactions and their relation to gut barrier function are displayed in Figure 2.

6. **Chronic stress dysregulates the HPA-axis and changes immune function**

Chronic psychological stress is known to dysregulate the immune system. These alterations are accompanied by low-grade inflammation, delayed wound healing and increased susceptibility to infectious diseases (79). Chronic stress leads to hypercortisolemia (77), long term permeability of barriers, endotoxemia and low-grade inflammation (our hypothesis and theory). Normally, the release of glucocorticoids puts a limit on the maximum activity of the immune system, however, chronic HPA-axis stimulation can result in glucocorticoid resistance at the level of the immune system, making it insensitive to its inhibitory and modulatory actions (2). This process is observed in several conditions (including conditions related to psychosocial stress), whereby immune cells from patients are less responsive to the inhibitory actions of glucocorticoids on cytokine release and cell proliferation after stimulation in vitro (80-83). In addition, chronic stress induces a shift in the production of type 1 cytokines towards type 2 cytokine production. It can be deducted that by this mechanism, the part of the immune system involved in the clearance of extracellular bacteria and bacterial toxins (the type 2 response) is prevented from being suppressed, protection against ongoing microbial infiltration (endotoxemia) is guaranteed, while the type 1 response, involved in clearance of intracellular pathogens (like viruses) is inhibited (71, 84).

7. **Life style-related factors induce endotoxemia**

The fact that stress increases barrier permeability and thereby enhances the availability of water, sodium and nutrients, makes sense from an evolutionary perspective. However, the question arises if the accompanied translocation of bacteria and their toxins should also be considered beneficial for the host. We speculate that when the composition of the microbiota is physiological, and barrier opening is short-lasting, acute stress will not produce low-grade inflammation. However, modern people suffer from new multi-factorial stressors, such as chronic psychosocial stress and the consumption of a “Western diet”, which constantly challenges the stress-axis, alters microbiota composition and thereby compromises intestinal barrier function. This next section discusses how modern lifestyle factors impact the gut-brain-immune-axis and promote endotoxemia, low-grade inflammation and its related diseases.
7.1. Gut microbiota modulate stress-axis and influence gut barrier function

Large differences in the composition of the gut microbiota and an overall reduction in microbial diversity are observed in Western populations when compared to traditional Hunter-gatherers or people from rural Africa (85, 86). These environment and diet-induced changes in gut microbiota have been connected to an increased susceptibility to chronic diseases, like IBD, obesity, type 1 and type 2 diabetes (87). The gut microbiota influences inflammatory (88) and metabolic processes (89) and has been shown to influence the development of the HPA-axis and immune system (90, 91). For example, exposure to LPS during developmental periods can exaggerate the HPA-axis and immune response to stress (92, 93), but also the absence of bacteria can induce these effects. Animals raised in germ-free environments showed an exaggerated HPA-axis response, which was normalized by colonization with fecal matter from specifically germ free animals or by the administration of the Gram-positive Bifidobacterium infantis (94). Vice versa, exposure to social stress changed the composition of the gut microbiota in mice (95, 96) and prenatal stress altered the microbiome in rhesus monkeys by reducing the overall numbers of the Gram-positive Bifidobacteria and Lactobacilli (97), indicating that chronic stress affected the composition of the gut microbiome. Stress influences gut motility, secretions, and mucin production, thereby altering the habitat of resident bacteria, promoting changes in the composition of the gut microbiome (98) and allowing the growth of pathogenic bacteria (99).

Increasing evidence supports an important role for microbiota on the homeostasis of the intestinal barrier. Certain strains of the Gram-positive Lactobacilli decreased intestinal permeability in several animal and human disease models (78, 100). Bifidobacterium infantis reduced intestinal permeability (as assessed by 70-kDa fluorescein isothiocyanate–dextran transmucosal flux) and ameliorated symptoms in a neonatal necrotizing enterocolitis mouse model (101). Further evidence indicating the influence of the gut microbiota on intestinal permeability was presented in detoxifying alcoholic-dependent subjects: Lower levels of Ruminococcaceae and higher abundance of Lachnospiraceae (Dorea) and Blautia were associated with increased intestinal permeability (102). In addition, higher levels of certain pathogenic bacteria can increase intestinal permeability by disrupting the epithelial barrier and triggering cell death and inflammation. These bacteria have the ability to bind and/or translocate through endothelial and microfold cells and have been shown to secrete toxins or other effector molecules via specialized secretion systems. Although the exact mechanisms are not well described, most pathogenic gut bacteria including Escherichia coli, Helicobacter pylori, Staphylococcus aureus, Cholera Pseudomonas fluorescens, Pseudomonas eruginosa, Yersinia enterocolitica, Campylobacter jejuni and Salmonella typhimurium alter paracellular permeability by disassembling tight junctions and generating cytoskeleton changes by increasing inflammation (reviewed by Barreau et al. (103)). As an example, a strain of
*Escherichia coli*, normally present in the human gut, induced focal leaks in colonic epithelial monolayers and in rat distal colon by using α-hemolysin, allowing for their paracellular translocation across the epithelial layer (104).

### 7.2. High-caloric and high-fat diets induce inflammation and increase circulating endotoxin levels

Compared to ealthy individuals, patients suffering from obesity have higher circulating endotoxin levels together with greater levels of circulating pro-inflammatory cytokines and insulin resistance (105). Food intake can produce postprandial immune activation and elevate endotoxin levels when a meal is high in calories (106) or has a high fat content (6, 107-109).

Rodents fed a 4-week high-fat diet (72% fat) showed a constant elevation in circulating endotoxin levels, while in control animals endotoxin levels only increased during feeding hours. Furthermore, a high-fat diet produced fasting glycaemia, insulin resistance, general weight gain and weight gain of the liver and visceral and subcutaneous adipose tissue. In addition, adipose tissue F4/80-positive cells (indicating the infiltration of macrophages), markers of inflammation and liver triglyceride content were increased. Interestingly, almost similar effects were observed in mice subcutaneously infused with LPS (resulting in similar circulating LPS levels as observed in the high-fat fed mice). These effects were mediated by TLR4, since mice lacking CD14, which is important for the recognition of LPS to this receptor, showed a delayed response to a high-fat diet or LPS injections (107).

In healthy humans a 910 calories high-fat and high carbohydrate meal resulted in increased circulating endotoxin levels and elevated levels of LBP in parallel with higher inflammatory markers and increased protein expression of TLR2 and TLR4 in isolated leukocytes. A meal high in fruits and fibre did not induce these effects (108). Plasma endotoxin levels, pro-inflammatory markers and leukocyte TLR4 expression increased after the intake of cream (300 calories), while the intake of 300 calories of glucose resulted only in a pro-inflammatory response and the intake of orange juice and water showed none of these effects (110). In healthy individuals, plasma endotoxin levels increased about 50% after the intake of a high-fat meal (900 calories) (6) and 4 weeks consumption of a Western-style diet raised plasma endotoxin activity levels by 71% (111).

How exactly the intake of a high-caloric meal increases circulating endotoxin levels is still unclear but has been explained by several mechanisms (reviewed by Kelly et al., 2012 (112). One of these suggested mechanisms is that the introduction of a high-fat diet modulates the expression of genes involved in the barrier function in epithelial cells, thereby directly compromising the integrity of the tight junction (113). Another explanation could be that a
high-caloric/high-fat meal induces high levels of insulin and leptin, hormones that directly activate the SNS (114, 115). Moreover insulin enhances SGLT1 mediated glucose absorption (116). Activation of the SGLT1 and the SNS leads to increased permeability of the gut barrier, which may induce the observed post-prandial endotoxemia (our hypothesis and theory).

7.3. Gliadin compromises the integrity of tight junctions
The intake of wheat and other cereal grains has been implicated in the development of inflammation-related diseases, by inducing inflammation and increasing intestinal permeability (40). Gliadin, a component of gluten, has been demonstrated to increase permeability in human Caco-2 intestinal epithelial cells by reorganizing actin filaments and altering expression of junctional complex proteins (117). Several studies by the group of Fasano et al. show that the binding of gliadin to the chemokine receptor CXCR3 on epithelial IEC-6 and Caco2 cells releases zonulin, a protein that directly compromises the integrity of the junctional complex (118, 119).

7.4. Alcohol consumption increases intestinal permeability
Alcohol consumption is an important risk factor for disease and is one of the major causes of chronic liver disease. Increased intestinal permeability has been observed during chronic alcohol consumption. In an animal model of chronic alcoholic liver disease, alcohol feeding for 8 weeks increased intestinal permeability (120). In humans, alcohol dependence induced changes in the gut microbiota composition that were associated with increased intestinal permeability (102). Furthermore, increased intestinal permeability and higher circulating endotoxin levels were observed in patients with chronic alcohol abuse (121-123).

7.5. Exercise induced heat-stress increases intestinal permeability
Exercise increases body temperature, reduces intestinal blood flow (reallocated to the muscles and cardiac system) and increases intestinal permeability by activating the SNS and HPA-axis. Already in 1992, Oktedalen et al. (124) showed that marathon runners displayed a significant increase in intestinal permeability. In addition, studies have indicated that strenuous exercise induced higher circulating endotoxin levels and activated the immune system (125-128). Further evidence of exercise- and heat-induced increased intestinal permeability, leading to gastrointestinal complaints in people engaging in physical activity, has been recently reviewed (129).
Endotoxemia is associated with diseases related to chronic inflammation

Multiple human studies have emerged that find associations with NCDs and markers of endotoxemia. Even aging, associated with higher sympathetic nerve activity (130) and higher circulating inflammatory mediators like IL-6, has been linked to higher plasma concentration of LPS and LBP (131). In further support of our theory, in this section an overview is given of human studies finding changes in levels of endotoxin or endotoxin-related markers in NCDs (Table 1).

8.1. Metabolic syndrome

Metabolic syndrome is accompanied by an increased risk for NAFLD, obesity, type 2 diabetes and cardiovascular diseases. All of these conditions are related to and even predicted by an increased sympathetic activity (132) and a dysregulated HPA-axis (133). Higher circulating endotoxin and LBP levels are associated with risk factors of the metabolic syndrome, like insulin resistance, obesity, dyslipidemia and chronic inflammation (134-137). Patients suffering from NAFLD exhibited significantly higher serum endotoxin levels in contrast to healthy controls (14). Farhardi et al. (138) indicated that elevated plasma endotoxin levels in these patients were related to an impaired intestinal barrier function, because, only in the patient group was the intake of a permeability stressor (aspirin) shown to increase the 0–24 h urinary excretion of sucralose (a marker of whole-gut permeability). Furthermore, augmented plasma LBP levels in concert with increased plasma levels of TNF-α were observed in obese NAFLD patients compared to healthy controls (139).

Elevated circulating levels of endotoxin and LBP were detected in type 2 diabetics (12, 135, 140-142). Compared to healthy controls, obese individuals and type 2 diabetics showed higher endotoxin levels after the intake of a high-fat meal. Increased endotoxin levels were observed in all challenged individuals, yet higher endotoxin levels were seen in individuals suffering from metabolic illnesses, suggesting an increased intestinal permeability in these patients (143). This was further indicated by a recent study showing that increased serum levels of endotoxin, IL-6 and TNF-α are found in type 2 diabetic patients compared to healthy individuals. The level of endotoxin was positively related to zonulin, a marker for intestinal permeability (12).

A large cohort of patients with coronary artery disease identified increased serum LBP levels to be associated with total and cardiovascular mortality (144). Moreover, circulating LBP levels were associated with carotid intima media thickness (a marker of atherosclerosis), obesity, insulin resistance and high sensitive CRP (145).
Patients suffering from chronic heart failure with aggravated renal function showed increased circulating endotoxin levels and an impairment of the intestinal barrier (11). Wiedermann et al. (146), showed that subjects with the highest levels of circulating endotoxin (90th percentile) had a 3-fold increased risk of incident atherosclerosis. Higher serum endotoxin and pro-inflammatory cytokine concentrations were seen in patients with edematous chronic heart disease compared to stable patients and healthy controls. Intriguingly, after short-term diuretic treatment, circulating endotoxin concentrations decreased in edematous patients (147). Diuretic treatment (like angiotensin-converting enzyme (ACE) inhibitors) ameliorated intestinal inflammation, perhaps by impacting on intestinal permeability through interference with the renin-angiotensin-aldosterone system. Several components of this system (renin, ACE and angiotensin II) have been shown to stimulate pro-inflammatory pathways (44, 148).

8.2. IBD
Ulcerative colitis and Crohn’s disease are intestinal inflammatory disorders, also known as IBD, which have been causally linked to chronic psychological stress (149), altered immune function, changes in the gut microbiota, increased intestinal permeability and endotoxemia (150). For example, increased plasma endotoxin and LBP levels were measured in both patient groups, but were more pronounced in patients with active disease compared to inactive disease and were associated with disease severity (151). In addition, detectable plasma endotoxin levels and higher plasma levels of LBP were more frequently observed in IBD patients compared to controls (15, 152) and were correlated with disease severity and circulating TNF-α levels (153).

8.3. Psychiatric diseases
Over the last decade, the role of the gut-brain axis has emerged as an important mediator in the development of psychiatric and mood disorders (154). Moreover, higher endotoxin levels and intestinal barrier dysfunction are observed in several of these conditions. For example, Parkinson’s patients exhibited increased total intestinal permeability and a more intense staining for Escherichia coli LPS and oxidative stress markers in intestinal sigmoid mucosa samples. However, in these patients endotoxin levels resembled control samples and serum LBP concentrations were lower compared to healthy individuals (155). Higher serum endotoxin levels are associated with severe autism, sporadic amyotrophic lateral sclerosis and Alzheimer’s disease (13, 156). Furthermore, increased IgA and IgM responses against LPS of commensal bacteria were seen in chronic fatigue syndrome (10) and depression (9). Intriguingly, chronic oral infection of periodontitis was associated with Alzheimer’s disease where higher antibody levels against oral pathogens were observed years before the onset of symptoms in people suffering from Alzheimer’s disease (157), suggesting there was an
increased translocation of bacteria and/or bacterial toxins from the mouth into the bloodstream.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Disease</th>
<th>Marker(s) of endotoxemia</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>133</td>
<td>Metabolic syndrome</td>
<td>Serum LPS</td>
<td>LPS levels correlated positively with symptoms of metabolic syndrome</td>
</tr>
<tr>
<td>133</td>
<td>Obesity-related insulin resistance</td>
<td>Serum LBP</td>
<td>LBP levels increased</td>
</tr>
<tr>
<td>134</td>
<td>Nonalcoholic fatty liver disease</td>
<td>Serum LBP</td>
<td>LBP levels only increased in patients with metabolic syndrome</td>
</tr>
<tr>
<td>135</td>
<td>Obesity</td>
<td>Plasma LBP</td>
<td>LBP levels increased</td>
</tr>
<tr>
<td>136</td>
<td>Nonalcoholic fatty liver disease</td>
<td>Serum LPS</td>
<td>LPS levels increased</td>
</tr>
<tr>
<td>137</td>
<td>Coronary artery disease</td>
<td>Serum LBP</td>
<td>LBP levels increased</td>
</tr>
<tr>
<td>138</td>
<td>Coronary artery disease</td>
<td>Serum LBP</td>
<td>LBP levels increased</td>
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<tr>
<td>139</td>
<td>Coronary artery disease</td>
<td>Serum LBP</td>
<td>LBP levels increased</td>
</tr>
<tr>
<td>140</td>
<td>Hypertension</td>
<td>Serum LBP</td>
<td>LBP levels increased</td>
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<td>141</td>
<td>Chronic heart failure (non-cardiogenic)</td>
<td>Plasma LPS</td>
<td>LPS levels increased</td>
</tr>
<tr>
<td>142</td>
<td>Chronic heart failure (cardiogenic)</td>
<td>Serum LPS, serum IgM/IgG against oral bacteria</td>
<td>LPS levels increased, no differences in IgM/IgG levels</td>
</tr>
<tr>
<td>143</td>
<td>Chronic heart failure (cardiogenic)</td>
<td>Plasma LBP</td>
<td>LBP levels increased</td>
</tr>
<tr>
<td>144</td>
<td>Idiopathic inflammatory bowel disease (IBD)</td>
<td>Serum LPS, LBP, sCD14</td>
<td>LPS, LBP, sCD14 levels increased</td>
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<tr>
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<td>Idiopathic inflammatory bowel disease (IBD)</td>
<td>Plasma LPS, LBP, sCD14, mCD14, and mCD14</td>
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<tr>
<td>146</td>
<td>Parkinson’s disease</td>
<td>Serum LBP, E. coli LPS infiltration in intestinal tissue</td>
<td>LPS levels decreased, increased LPS infiltration in intestinal tissue</td>
</tr>
<tr>
<td>147</td>
<td>Parkinson’s disease</td>
<td>Plasma LPS, sCD14</td>
<td>LPS levels increased in non-cardiogenic vs. non-cardiogenic patients No differences between all patients vs. controls</td>
</tr>
<tr>
<td>148</td>
<td>Sporadic amyotrophic lateral sclerosis,</td>
<td>Plasma LPS, endoCAMS</td>
<td>LPS and endoCAMS levels increased with disease severity</td>
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<td>149</td>
<td>Alzheimer’s disease</td>
<td>Plasma LPS, sCD14</td>
<td>LPS levels increased in non-cardiogenic vs. non-cardiogenic patients No differences between all patients vs. controls</td>
</tr>
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<td>150</td>
<td>Depression</td>
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<td>Chronic fatigue syndrome</td>
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<tr>
<td>152</td>
<td>Alzheimer’s disease</td>
<td>Serum LPS, IgG against oral bacteria</td>
<td>IgG levels increased</td>
</tr>
</tbody>
</table>

Table 1. Associations found between markers of endotoxemia and disease.
9 Conclusion

Chronic low-grade inflammation is an eminent feature of NCDs. In addition, many studies report increased circulating endotoxin levels and increased gut permeability in patients suffering from these conditions. As reviewed in this paper, stress-induced increases in intestinal permeability, in combination with modern life-style factors, raise the possibility of translocation of bacteria and/or their toxins across the more permeable gut barrier. The resulting, long lasting, endotoxemia should be considered much more than just a risk factor for chronic disease; it could be a cause. Notwithstanding the fact that the exact origin and sequence of events involved in development of NCDs remain to be unsolved, evidence indicates that a disrupted barrier function in parallel with elevated circulating endotoxin levels may underlie disease onset and progression. For this reason, applying therapies aimed at restoring intestinal barrier function, lifestyle changes and stress management should be considered as an important strategy in preventing and attenuating the pro-inflammatory state observed in NCDs.

Conflict of interest statement
The authors declare no conflict of interest.

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References


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Figure 1. MLC phosphorylation increases intestinal permeability.

Activation of the SNS stimulates intestinal permeability by increasing the activity of SGLT1 on epithelial cells. Activation of SGLT1 is paralleled by MLC phosphorylation by MLCK, inducing actomyosin contraction and reorganization of the tight junction. The resulting increase in paracellular permeability raises the possibility of translocation of bacteria and/or their toxins across the more permeable gut barrier. Pro-inflammatory cytokines produced by activated immune cells residing in the lamina propria further increase intestinal permeability by activating MLCK. JC, junctional complex.
Stressors, including inflammatory mediators, activate the SNS and HPA-axis. Activation of the HPA-axis stimulates the neurons in the paraventricular nucleus of the hypothalamus to secrete CRH and AVP that trigger the release of ACTH from the anterior pituitary, resulting in the secretion of corticosteroids from the adrenal cortex. CRH has been shown to affect intestinal permeability. SNS activation results in the release of catecholamines from the adrenal medulla. The intestinal wall is innervated by adrenergic sympathetic nerve fibers that upon stimulation increase water, sodium and glucose absorption, paralleled by increased intestinal permeability. The resulting increase in translocation of endotoxin across the intestinal barrier can stimulate immune cells in the underlying lamina propria to secrete pro-inflammatory cytokines and prostaglandins like PgE2. Inflammatory mediators communicate with the brain by stimulating afferent sensory nerve fibers, by entering the brain via the circumventricular organs or by binding to cerebral blood vessel endothelium. Continuous stress-induced impairment of the intestinal barrier creates a vicious circle whereby inflammatory cytokines will persistently activate the SNS and HPA-axis resulting in barrier disruption, increased endotoxin translocation and a pro-inflammatory state.
Paragraph 1.5. The dietary intake of wheat and other cereal grains and their role in inflammation. de Punder K, Pruimboom L. Nutrients 2013;5:771-87.

The Dietary Intake of Wheat and other Cereal Grains and their Role in Inflammation

Karin de Punder ¹, Leo Pruimboom¹ ², *

1. University of Girona, Plaça Sant Domènec, 3 Edifici Les Àligues, 17071 Girona, Spain; E-Mail: k.d.punder@nki.nl
2. Uni for Life, Universität Graz, Beethovenstraße 9, 8010, Graz, Austria; E-Mail: udgleo@yahoo.es

Abstract

Wheat is one of the most consumed cereal grains worldwide and makes up a substantial part of the human diet. Although government supported dietary guidelines in Europe and the U.S.A advise individuals to eat adequate amounts of (whole) grain products per day, cereal grains contain "anti-nutrients", such as wheat gluten and wheat lectin, that in humans can elicit dysfunction and disease. In this review we discuss evidence from in vitro, in vivo and human intervention studies that describe how the consumption of wheat, but also other cereal grains, can contribute to the manifestation of chronic inflammation and auto-immune diseases by increasing intestinal permeability and initiating a pro-inflammatory immune response.

Keywords

cereal grains; coeliac disease; gluten; gliadin; inflammation; intestinal permeability; lectins; wheat; wheat germ agglutinin
1. Introduction

Inflammation is the response of the innate immune system triggered by noxious stimuli, microbial pathogens and injury. When a trigger remains or when immune cells are continuously activated an inflammatory response may become self-sustainable and chronic. Chronic inflammation has been associated with many medical and psychiatric disorders, including cardiovascular disease, metabolic syndrome, cancer, autoimmune diseases, schizophrenia and depression [1,2,3]. Furthermore, it is usually associated with elevated levels of pro-inflammatory cytokines and acute phase proteins, such as interferons (IFNs), interleukin (IL)-1, IL-6, tumor necrosis factor-α (TNF-α), and C-reactive protein (CRP). While clear peripheral sources for this chronic inflammation are apparent in some conditions (i.e. fat production of cytokines in the metabolic syndrome), in other disorders, such as major depression, the inflammatory source is not completely understood. Genetic vulnerability, psychological stress and poor dietary patterns have all been repeatedly implicated as being of significant importance in the development of an inflammatory phenotype [3,4,5]. Dietary factors associated with inflammation include a shift towards a higher n-6:n-3 fatty acid ratio [5] and a high intake of simple sugars [6]. Other substances in our daily food, like those found in wheat and other cereal grains, are also capable of activating pro-inflammatory pathways.

2. Wheat grain, gluten and disease

2.1. Wheat allergy and intolerance

The ingestion of wheat products has been reported to be responsible for IgE-mediated allergic reactions. Wheat-dependent exercise-induced anaphylaxis is a syndrome in which the ingestion of a product containing wheat followed by physical exercise can result in an anaphylactic response. Several proteins present in wheat, most notably gluten proteins have been shown to react with IgE in patients [7]. Other allergic responses that appear to be related to a range of wheat proteins include Bakers asthma, rhinitis and contact urticaria [7,8].

More common than wheat allergies are conditions involving wheat intolerance, including coeliac disease (CD), which is estimated to affect 1% of the population of Western Europe, and dermatitis herpetiformis, which has an incidence between about 2-fold and 5-fold lower than CD [9]. The close association between type 1 diabetes and CD [10] and the observation that autoimmune diseases seem to be more prevalent in coeliac patients and their relatives [11] associate the intake of wheat with several other conditions.
2.2. Wheat grain and gluten

Gluten is the main structural protein complex of wheat consisting of glutenins and gliadins. When wheat flour is mixed with water to form dough, the gluten proteins form a continuous network which provides the cohesiveness and viscoelasticity that allows dough to be processed into bread, noodles and other foods. The protein contents of wheat varies between 7-22% with gluten constituting about 80% of the total protein of the seed [9]. Glutenins are the fraction of wheat proteins that are soluble in dilute acids and are polymers of individual proteins. Prolamins are the alcohol soluble proteins of cereal grains and are specifically named gliadins in wheat. Gliadins are monomeric proteins and are classified into three groups: these are α/β-gliadins, γ-gliadins and ω-gliadins [7].

2.3. Gluten, gliadin and CD

Gliadin epitopes from wheat gluten and related prolamins from other gluten-containing cereal grains, including rye and barley, can trigger CD in genetically susceptible people. The symptoms of this disease are mucosal inflammation, small intestine villous atrophy, increased intestinal permeability and malabsorption of macro- and micronutrients. CD, a chronic inflammatory disorder mediated by T-cells, is preceded by changes in intestinal permeability and pro-inflammatory activity of the innate immune system. Gliadin immunomodulatory peptides can be recognized by specific T-cells, a process that can be enhanced by the deamidation of gliadin epitopes by tissue transglutaminases that convert particular glutamine residues into glutamic acid resulting in a higher affinity for HLA-DQ2 or DQ8 expressed on antigen presenting cells (APC) [10]. Serum antibodies, among which are antibodies against tissue transglutaminases, are also found in CD. The HLA-DQ2 or HLA-DQ8 is expressed in 99.4% of the patients suffering from CD [10], but interestingly enough there is a group HLA-DQ2/DQ8 negative patients suffering from gastrointestinal symptoms that respond well to a gluten-free diet. This group of “gluten-sensitive” patients does not have the CD serology and histopathology, but does present the same symptoms and shows improvements when following a gluten-free diet [12,13].

2.4. Gliadin and immunity

There are at least 50 gliadin epitopes that exert immunomodulatory, cytotoxic and gut permeating activities that can be partially traced back to different domains of α-gliadin. Where some immunomodulatory gliadin peptides activate specific T-cells, others are able to induce a pro-inflammatory innate immune response [10]. Stimulation of immune cells by gliadin is not only restricted to CD patients; the incubation of peripheral blood mononuclear cells (PBMC) from healthy HLA-DQ2 positive controls and CD patients with gliadin peptides stimulated the production of IL-23, IL-1β and TNF-α in all donors tested. Still the production of cytokines was significantly higher in PBMC derived from CD patients [14]. Similar results were obtained by
Lammers et al. [15] showing that gliadin induced an inflammatory immune response in both CD patients and healthy controls, yet IL-6, IL-13 and IFN-γ were expressed at significantly higher levels in CD patients. IL-8 production was only expressed in a subset of healthy and CD individuals after stimulation with a specific gliadin peptide and appeared to dependent on the CXCR3 chemokine receptor only in CD patients. Sapone et al. [16] showed that in a subset of CD patients, but not in gluten-sensitive patients (with 36% of the studied individuals in this group being HLA-DQ2/DQ8 positive), there is an increased IL-17 mRNA expression in the small-intestinal mucosa compared to healthy controls. The same group showed that in a subset of gluten sensitive patients (with 50% of the studied individuals being HLA-DQ2/DQ8 positive) there is a prevailing stimulation of cells of the innate immune system, while in CD both the innate and adaptive immune system are involved [13].

2.5. Gliadin and intestinal permeability

In order for gliadin to interact with cells of the immune system it has to overcome the intestinal barrier. Gliadin peptides cross the epithelial layer by transcytosis or paracellular transport. Paracellular transport occurs when intestinal permeability is increased, a feature that is characteristic for CD [17]. It is indicated by several studies that increased intestinal permeability precedes the onset of CD and is not just a consequence of chronic intestinal inflammation [18,19]. Gliadin has been demonstrated to increase permeability in human Caco-2 intestinal epithelial cells by reorganizing actin filaments and alter expression of junctional complex proteins [20]. Several studies by the group of Fasano et al. show that the binding of gliadin to the chemokine receptor CXCR3 on epithelial IEC-6 and Caco2 cells releases zonulin, a protein that directly compromises the integrity of the junctional complex [21,22]. Although zonulin levels were more up-regulated in CD patients, zonulin was activated by gliadin in all intestinal biopsies from both CD and non-CD patients [21,22], suggesting that gliadin can increase intestinal permeability also in non-CD patients, yet increased intestinal permeability was not observed in a group of gluten-sensitive patients [13].

3. Increased intestinal permeability

3.1. Increased intestinal permeability is associated with disease

Chronically increased intestinal permeability (or leaky gut syndrome) allows for the increased translocation of both microbial and dietary antigens to the periphery which can then interact with cells of the immune system. Shared amino acid motifs among exogenous peptides (HLA-derived peptides and self tissue) may produce cross reactivity through immunological mimicry, thereby disturbing immune tolerance in genetically susceptible individuals [23]. Not surprisingly, increased intestinal permeability has been associated with auto-immune diseases, such as type 1 diabetes [24], rheumatoid arthritis, multiple sclerosis [18], but also with
diseases related to chronic inflammation like inflammatory bowel disease [18,25], asthma [26], chronic fatigue syndrome and depression. The latter two conditions see patients with significantly greater values of serum IgA and IgM to LPS of gram-negative enterobacteria compared to controls, implying intestinal permeability is increased in these patients [27,28,29].

3.2. Intestinal barrier function and inflammation

The intestinal barrier allows the uptake of nutrients and protects from damage of harmful substances from the gut lumen. Macromolecules that can be immunogenic like proteins, large peptides but also bacteria and lectins can be endocytosed or phagocytosed by enterocytes forming the epithelial layer of the gut. Absorbed proteins generally will be entering the lysosomal route and will be degraded to small peptides. Normally, only small amounts of antigen pass the barrier by transcytosis and interact with the innate and adaptive immune system situated in the lamina propria. Highly specialized epithelial microfold (M) cells function as active transporters of dietary and microbial antigens from the gut lumen to the immune system where either a pro-inflammatory or tolerogenic immune response can be generated. The paracellular route is regulated by the junctional complex that allows the passage of water, solutes and ions, but under normal conditions provides a barrier to larger peptides and protein-sized molecules. When the barrier function is disrupted there is an increased passage of dietary and microbial antigens interacting with cells of the immune system [25,30] (Figure 1).

3.3. The role of zonulin signaling on intestinal permeability

Intestinal permeability is a measure of the barrier function of the gut which relates to the paracellular space surrounding the brush border surface of the enterocytes and the junctional complexes consisting of tight junctions, adherent junctions, desmosomes and gap junctions [31]. The junctional complexes are regulated in response to physiological and immunological stimuli, like stress, cytokines, dietary antigens and microbial products [31]. As mentioned before, zonulin, a protein identified as prehaptoglobulin-2 (the precursor of haptoglobin-2) is also a regulator of intestinal permeability. Haptoglobin-2, together with haptoglobin-1, is one of the two gene variants of the multifunctional protein haptoglobin and is associated with an increased risk for CD (homozygotes and heterozygotes) and severe malabsorption (homozygotes) [32,33]. The haptoglobin-2/zonulin allele has a frequency of about 0.6 in Europe and the U.S.A, but varies throughout the world depending on racial origin [34].
Increased intestinal permeability allows for the passage of microbial and dietary antigens across the epithelial layer into the lamina propria where these antigens can be taken up by APC and presented to T-cells. JC, junctional complex.

3.4. High zonulin levels are observed in auto-immune and inflammatory diseases

Zonulin signalling is proposed to cause rearrangements of actin filaments and induces the displacement of proteins from the junctional complex, thereby increasing permeability [18,32,35]. Gliadin peptides initiate intestinal permeability through the release of zonulin, thereby enabling paracellular translocation of gliadin and other dietary and microbial antigens, which by interacting with the immune system give rise to inflammation. In this manner a vicious cycle is created in which, as a consequence of the persistent presence of pro-inflammatory mediators, intestinal permeability will increase even further. High zonulin levels (together with increased intestinal permeability) have been observed in auto-immune and inflammatory diseases like CD, multiple sclerosis, asthma and inflammatory bowel disease and the haptoglobin polymorphism is associated with rheumatoid arthritis, ankylosing spondylitis, schizophrenia and certain types of cancer [32].

The zonulin inhibitor Larozotide acetate was tested in an inpatient, double-blind randomized placebo controlled trial. The group of CD patients in the placebo group that were exposed to...
gluten showed a 70% increase in intestinal permeability, while no changes were seen in the group exposed to Larazotide acetate. Also gastrointestinal symptoms were significant more frequent in the placebo group [32]. These results suggest that in CD patients, when intestinal barrier function is restored, autoimmunity will disappear while the trigger (gluten) is still there. Besides gliadin from wheat gluten, the lectin wheat germ agglutinin (WGA) has also been shown to stimulate cells of the immune system and increase intestinal permeability, as we will now further discuss.

4. **WGA**

4.1. **Dietary WGA**

Lectins are present in a variety of plants, especially in seeds, where they serve as defense mechanisms against other plants and fungi. Because of their ability to bind to virtually all cell types and cause damage to several organs, lectins are widely recognized as anti-nutrients within food [36]. Most lectins are resistant to heat and the effects of digestive enzymes and are able to bind to several tissues and organs in vitro and in vivo (reviewed by Freed 1991 [37]). The administration of the lectin WGA to experimental animals caused hyperplastic and hypertrophic growth of the small intestine, hypertrophic growth of the pancreas and thymus atrophy [36]. Lectin activity has been demonstrated in wheat, rye, barley, oats, corn and rice, however the best studied of the cereal grain lectins is WGA [38].

The highest WGA concentrations are found in wheat germ (up to 0.5 g/kg [39]). Although unprocessed wheat germ, like muesli, contains far higher amounts of active WGA than do processed wheat germ products, WGA activity is still apparent in several processed breakfast cereals as assessed by hemagglutination and bacterial agglutination assays [40,41]. A summary of the amount of active WGA in commonly consumed wheat derived products is listed in Table 1.

4.2. **WGA binds to cell surface glycoconjugates**

WGA binds to N-glycolylneuraminic acid (Neu5Ac), the sialic acid predominantly found in humans [42], allowing it to adhere to cell surfaces like the epithelial layer of the gut. The surface of many prokaryotic and eukaryotic cells are covered with a dense coating of glycoconjugates, also named glycocalyx. Sialic acids are a wide family of nine-carbon sugars that are typically found at the terminal positions of many surface exposed glycoconjugates and function for self recognition in the vertebrate immune system, but they can also be used as binding target for pathogenic extrinsic receptors and molecular toxins [43,44,45]. WGA binding to Neu5Ac of the glycocalyx of human cells (and pathogens expressing Neu5Ac) allows for cell entry and could disturb immune tolerance by evoking a pro-inflammatory immune response (discussed below).
4.3. WGA and immunity

WGA induces inflammatory responses by immune cells. For example, WGA has been shown to trigger histamine secretion and granule extrusion from non-stimulated rat peritoneal mast cells [46], induce NADP-oxidase activity in human neutrophils [47] and stimulate the release of the cytokines IL-4 and IL-13 from human basophils [48]. In human PBMC, WGA induced the production of IL-2, while simultaneously inhibiting the proliferation of activated lymphocytes [49]. WGA stimulated the secretion of IL-12, in a T and B-cell independent manner in murine spleen cells. IL-12, in turn, activated the secretion of IFN-γ by T or natural killer cells [50]. In murine peritoneal macrophages WGA induced the production of the pro-inflammatory cytokines TNF-α, IL-1β, IL-12 and IFN-γ [51]. Similar results have been observed in isolated human PBMC, given that nanomolar concentrations of WGA stimulated the release of several pro-inflammatory cytokines. In the same study a significant increase in the intracellular accumulation of IL-1β was measured in monocytes after WGA exposure [52]. These results indicate that when delivered in vitro WGA is capable of directly stimulating monocytes and macrophages, cells that have the ability to initiate and maintain inflammatory responses. Monocytic cells have been shown to engulf WGA via receptor-mediated endocytosis or by binding to non-receptor glycoproteins [53].

Human data showing the influence of WGA intake on inflammatory markers are lacking, however, antibodies to WGA have been detected in the serum of healthy individuals [54]. Significantly higher antibody levels to WGA were measured in patients with CD compared to patients with other intestinal disorders. These antibodies did not cross-react with gluten antigens and could therefore play an important role in the pathogenesis of this disease [55].
Table 1. Amount of active WGA in wheat derived products

<table>
<thead>
<tr>
<th>Wheat derived products</th>
<th>WGA µg/g (±SD)</th>
<th>Reference source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat germ</td>
<td>300 (±35)</td>
<td>Vincenzi et al., 2000 [56]</td>
</tr>
<tr>
<td>Wheat germ</td>
<td>100 - 500</td>
<td>Peumans and Van Damme, 1996 [39]</td>
</tr>
<tr>
<td>Semolina&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.0 (±1.0) – 10.7 (±1.5)</td>
<td>Matucci et al., 2004 [57]</td>
</tr>
<tr>
<td>Flour&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.3 (±0.7) – 4.4 (±1.0)</td>
<td></td>
</tr>
<tr>
<td>Wholemeal flour&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.5 (±2.5) – 50 (±5.5)</td>
<td></td>
</tr>
<tr>
<td>Pasta&lt;sup&gt;a&lt;/sup&gt;</td>
<td>≤ 0.4 (±0.2) – 3.2 (±0.2)</td>
<td></td>
</tr>
<tr>
<td>Pasta cooked&lt;sup&gt;a&lt;/sup&gt;</td>
<td>≤ 0.3 (±0.2)</td>
<td></td>
</tr>
<tr>
<td>Wholemeal pasta (enriched with wheat germ)</td>
<td>40 (±2.7)</td>
<td></td>
</tr>
<tr>
<td>Wholemeal pasta (enriched with wheat germ) cooked</td>
<td>Not detectable</td>
<td></td>
</tr>
<tr>
<td>Wholemeal pasta&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0 – 5.7 (±0.2)</td>
<td></td>
</tr>
<tr>
<td>Wholemeal pasta cooked&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Not detectable</td>
<td></td>
</tr>
<tr>
<td>Breakfast cereals&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13 - 53</td>
<td>Ortega-Barria et al., 1994 [41]</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values are obtained from more than one product and from different manufacturers.

4.4. WGA and intestinal permeability

After ingestion, WGA is capable of crossing the intestinal barrier. In animal models, WGA has been shown to reach the basolateral membrane and walls of the small blood vessels in the subepithelium of the small intestine [36]. WGA can be phagocytosed by binding to membrane non-receptor glycoproteins, a process that has been observed in Caco-2 cells [58]. WGA can also be endocytosed by antigen sampling M-cells [59,60] or by enterocytes via binding to epidermal growth factor receptors [61]. Another possible route for lectin entry into the periphery is by paracellular transport, a process that can be further aggravated by the binding of gliadin to the chemokine receptor CXR3 on enterocytes.

WGA itself has been found to affect enterocyte permeability. Investigations by Dalla Pellegrina et al. [52] showed in vitro that exposure to micromolar concentrations of WGA impairs the integrity of the intestinal epithelial layer, allowing passage of small molecules, like lectins. At the basolateral side of the epithelium, WGA concentrations in the nanomolar range induced the secretion of pro-inflammatory cytokines by immune cells [52]. This may further affect the integrity of the epithelial layer, heightening the potential for a positive feedback loop between WGA, epithelial cells and immune cells. When combined, these mechanisms are likely able to significantly increase the percentage of consumed WGA that can cross the epithelial layer.
compared to the low percentage of WGA crossing by means of transcytosis (0.1%) alone [52]. This suggests that together with gliadin, WGA can increase intestinal permeability, resulting in an increase of translocating microbial and dietary antigens interacting with cells of the immune system.

5. Animal data on cereal grain intake

There are two rodent models of spontaneous type 1 diabetes: the non-obese-diabetic (NOD) mouse and the diabetes-prone BioBreeding (BBdp) rat. In these animals a cereal-based diet containing wheat induced the development of type 1 diabetes, while animals fed a hypoallergenic diet (gluten free) or a hypoallergenic diet supplemented with casein showed a decreased incidence and a delayed onset of this disease. BBdp rats fed a cereal-based diet showed increased intestinal permeability and a significant increase in the percentage of IFN-γ producing Th1 lymphocytes in the mesenteric lymph nodes in the gut [30]. Compared to animals fed a hypoallergenic diet, NOD mice fed a wheat-based diet expressed higher mRNA levels of the pro-inflammatory cytokines IFN-γ and TNF-α and the inflammatory marker inducible NO synthase in the small intestine. While these diet-induced changes in gut-wall inflammatory activity did not translate to increased cytokine mRNA in Peyers patches, structures that contribute to immune regulation to exogenous antigens, it is possible that the gut-signal may promote systemic inflammation via other mechanisms, such as activating intraepithelial lymphocytes and mesenteric lymph node cells [62]. These in vivo results show that in two rodent models of spontaneous type 1 diabetes a cereal containing diet induces the (early) onset of disease and increases markers of inflammation. In addition, Chignola et al. [63] have shown in rats that a WGA-depleted diet was associated with reduced responsiveness of lymphocytes from primary and secondary lymphoid organs after in vitro stimulation and attenuated spontaneous proliferation when compared to lymphocytes from rats fed a WGA-containing diet, indicating the stimulatory effect of WGA on cells of the immune system.

6. Human studies on cereal grain intake and inflammation

6.1. Human epidemiological data on cereal grain intake and inflammation

Observational prospective and cross-sectional studies show that the intake of whole grain products is associated with reduced risks for developing type 2 diabetes, cardiovascular diseases, obesity and some types of cancer [64]. Inflammation is associated with these conditions and some studies have shown that associations between the intake of whole grains and decreased inflammatory markers (CRP, IL-6) are found [65]. Intervention studies, however, do not demonstrate a clear effect of the intake of whole grains on inflammation [66,67,68,69,70,71] and it could therefore be that other components in the diet modulate the immune response.
It has been shown that the intake of whole grains is associated with healthier dietary factors and a healthier lifestyle in general. In a Scandinavian cross sectional study, the intake of whole grains was directly associated with the length of education, the intake of vegetables, fruits, dairy products, fish, shellfish, coffee, tea and margarine and inversely associated with smoking, BMI and the intake of red meat, white bread, alcohol, cakes and biscuits [72]. Good quality epidemiological studies attempt to control for these confounding factors, but with the consequence that associations are attenuated or become insignificant.

6.2. Human intervention trials on cereal grain intake and inflammation

To really estimate the causal relationship of cereal grain intake and inflammation, intervention trials provide us with better evidence. Wolever et al. [71] showed that a diet with a low glycemic index (containing whole grains) compared to high (containing refined grain products), resulted in sustained reductions in postprandial glucose and CRP levels on the long-term in patients with type 2 diabetes treated with diet alone. A refined grain is a whole grain that has been stripped of its outer shell (fiber) and its germ, leaving only the endosperm, resulting in lower levels of macro- and micronutrients and a higher dietary glycemic index for refined grains compared to whole grains. Refined wheat products contain less WGA, but still contain a substantial amount of gluten. It should be noted that whole grains contain phytochemicals, like polyphenols, that can exert anti-inflammatory effects which could possibly offset any potentially pro-inflammatory effects of gluten and lectins [73].

The substitution of whole grain (mainly based on milled wheat) for refined grains products in the daily diet of healthy moderately overweight adults for 6 weeks did not affect insulin sensitivity or markers of lipid peroxidation and inflammation [66]. Consistent with these finding are the results of Brownlee et al. [67], who showed that infrequent whole-grain consumers, when increasing whole grain consumption (including whole wheat products), responded with no improvements of the studied biomarkers of cardiovascular health, including insulin sensitivity, plasma lipid profile and markers of inflammation. The substitution of refined cereal grains and white bread with 3 portions of whole wheat food or 1 portions of whole wheat food combined with 2 servings of oats significantly decreased the systolic blood pressure and pulse pressure in middle-aged, healthy, overweight men and women, yet none of the interventions significantly affected systemic markers of inflammation [70]. In obese adults suffering from metabolic syndrome there were significantly greater decreases in CRP and the percentage of body fat in the abdominal region in participants consuming whole grains compared to those consuming refined grains. It must be noted that both diets were hypocaloric (reduced by 500 kcal/d) [69]. Most of the intervention studies mentioned above attempted to increase whole-grain intake and were
using refined grain diets as controls, thereby making it very difficult to draw any conclusions on the independent role of cereal grains in disease and inflammation.

6.3. Health effects of the paleolithic diet

There are few studies that investigate the influence of a paleolithic type diet comprising lean meat, fruits, vegetables and nuts, and excluding food types, such as dairy, legumes and cereal grains, on health. In domestic pigs the paleolithic diet conferred higher insulin sensitivity, lower CRP and lower blood pressure when compared to a cereal based diet [74]. In healthy sedentary humans the short-term consumption of a paleolithic type diet improved blood pressure and glucose tolerance, decreased insulin secretion, increased insulin sensitivity and improved lipid profiles [75]. Glucose tolerance also improved in patients suffering from a combination of ischemic heart disease and either glucose intolerance or type 2 diabetes that were advised to follow a paleolithic diet. Control subjects who were advised to follow a Mediterranean-like diet based on whole grains, low-fat dairy products, fish, fruits and vegetables did not significantly improve their glucose tolerance despite decreases in weight and waist circumference [76]. Similar positive results on glycemic control were obtained in diabetic patients when the paleolithic diet was compared with the diabetes diet. Participants were on each diet for 3 months where the paleolithic diet resulted in a lower BMI, weight and waist circumference, higher mean HDL, lower mean levels of hemoglobin A1c, triacylglycerol and diastolic blood pressure, yet levels of CRP were not significantly different [77]. Although the paleolithic diet studies are small, these results suggest that, together with other dietary changes, the withdrawal of cereal grains from the diet has a positive effect on health. Nevertheless, because these studies are confounded by the presence or absence of other dietary substances and by differences in energy and macronutrient intake, factors that could all affect markers of inflammation, it is difficult to make a concise statement on the impact of cereal grains on these health outcomes.

6.4. Rechallenge trial of effects of dietary gluten

One human intervention study specifically focused on the effects of dietary gluten on inflammation. Biesiekierski et al. [12] undertook a double-blind randomized, placebo-controlled rechallenge trial to investigate the influence of gluten in individuals with irritable bowel syndrome but without clinical features of CD, who reached satisfactory levels of symptom control with a gluten-free diet. After screening the participants, about 50% of the individuals in both the gluten and placebo group were HLA-DQ2 and/or HLA-DQ8 positive. Participants received either gluten or placebo together with a gluten-free diet for 6 weeks. End-points in the study were symptom assessments and biomarkers of inflammation and intestinal permeability. The patients receiving gluten reported significantly more symptoms compared to the placebo group. The most striking outcome of this study was that for all the
endpoints measured there were no differences in individuals with or without HLA-DQ2/DQ8, indicating that the intake of gluten can cause symptoms also in individuals without this specific HLA-profile. No differences in biomarkers for inflammation and intestinal permeability were found between both groups, however, inflammatory mediators have been implicated in the development of symptoms in patients with irritable bowel syndrome [78]. It could therefore be that the markers used to measure inflammation and intestinal permeability were not sensitive enough to detect subtle changes on the tissue level.

7. **Conclusion**

In the present review we describe how the daily consumption of wheat products and other related cereal grains could contribute to the manifestation of chronic inflammation and autoimmune diseases. Both *in vitro* and *in vivo* studies demonstrate that gliadin and WGA can both increase intestinal permeability and activate the immune system. The effects of gliadin on intestinal permeability and the immune system have also been confirmed in humans. Other cereal grains containing related prolamins and lectins have not been so extensively studied and therefore more research investigating their impact on intestinal permeability and inflammation is required. It would be interesting to further elucidate the role of other prolamins on zonulin release and intestinal permeability.

In CD and gluten-sensitive individuals adverse reactions to the intake of wheat, rye and barley are clinically apparent, however, it is important to gain better insights on the effects of the consumption of these cereal grains in other groups of patients and in healthy individuals. It would be of high interest to investigate the effects of the withdrawal of cereal grain products from the diet on inflammatory markers and intestinal permeability in healthy subjects and patients suffering from inflammation-related diseases and measure the same parameters in a rechallenge trial. Ideally, in such an intervention study, the diet must be completely controlled and combined with the appropriate substitution of foods in the cereal grain-deprived diet so that small dietary variations and alterations in energy intake can be avoided and cannot potentially influence inflammatory markers.

Until now, human epidemiological and intervention studies investigating the health-effects of whole grain intake were confounded by other dietary and lifestyle factors and therefore well designed intervention studies investigating the effects of cereal grains and their individual components on intestinal permeability and inflammation are warranted.
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Conflict of Interest
The authors declare no conflict of interest.
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1.6. Lactase persistence and augmented salivary alpha-amylase gene copy numbers might have been selected by the combined toxic effects of gluten and (food born) pathogens.


Lactase persistence and augmented salivary alpha-amylase gene copy numbers might have been selected by the combined toxic effects of gluten and (food born) pathogens

Leo Pruimboom MSc, Tom Fox, Frits A.J. Muskiet PhD
1. University of Gerona (Spain), Passeig Devesa 41, Gerona, Spain;
2. Laboratory Medicine, University of Groningen, University Medical Center Groningen (UMCG), P.O. Box 30.001 Groningen, The Netherlands.

Keywords
Lactose, amylase, gene copy number, lactase, starch, Rotavirus, mycotoxins, Aspergillus, Tuberculosis, glucose transporter, evolution, gluten, dairy, milk, cereals, fungus

Corresponding author
Leo Pruimboom
Avd del Mar Residencial Las Caletas G4
35508 Costa Teguise, Spain
udgleo@yahoo.es
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References
Abstract

Various positively selected adaptations to new nutrients have been identified. Lactase persistence is among the best known, conferring the ability for drinking milk at post weaning age. An augmented number of amylase gene (AMY1) copies, giving rise to higher salivary amylase activity, has been implicated in the consumption of starch-rich foods. Higher AMY1 copy numbers have been demonstrated in populations with recent histories of starchy-rich diets. It is however questionable whether the resulting polymorphisms have exerted positive selection only by providing easily available sources of macro and micronutrients. Humans have explored new environments more than any other animal. Novel environments challenge the host, but especially its immune system with new climatic conditions, food and especially pathogens. With the advent of the agricultural revolution and the concurrent domestication of cattle came new pathogens. We contend that specific new food ingredients (e.g. gluten) and novel pathogens drove selection for lactase persistence and higher AMY gene copy numbers. Both adaptations provide ample glucose for activating the sodium glucose-dependent co-transporter 1 (SGLT1), which is the principal glucose, sodium and water transporter in the gastro-intestinal tract. Their rapid uptake confers protection against potentially lethal dehydration, hyponatraemia and ultimately multiple organ failure. Oral rehydration therapy aims at SGLT1 activity and is the current treatment of choice for chronic diarrhoea and vomiting. We hypothesize that lifelong lactase activity and rapid starch digestion should be looked at as the evolutionary covalent of oral rehydration therapy.
Introduction

Adaption to new environmental challenges aims at phenotypic adjustments either by epigenetic (rapid, but labile) and genetic (slow, but robust) changes through processes such as epimutation and de novo mutation, the latter e.g. by variation of gene copy number (GCN) (1, 2). Many environmental factors have shaped the human genome, including climate, diet and microbial load (3). Although the first two certainly exerted selective pressure on humans, the main selective pressure seems to derive from pathogens because of their high lethality (4). Fumagalli et al. (4) identified a surprisingly high number of more than one hundred genes carrying signatures of a pathogenic environment and presenting as an increase in allele frequency (GCN). Conversely, for dietary regimes and climatic conditions, no gene shows a similar correlation between an environmental factor and GCN (4). Other authors have shown that climate and diet do act as selective pressure factors in humans (5, 6), but the majority of the literature indicates that it are pathogens and the pathogenic load that should be considered as the principal environmental factors causing selective pressure in humans (7,8).

Two recent changes in the human diet i.e. the inclusion of dairy products and the increased intake of starch, have been related with genetic adaptations, i.e. lactase (also known as lactase-phlorizin hydrolase; LPH) persistence (9) and salivary amylase (AMY1) GCN (10), respectively. Both adaptations are ascribed to toxicosis as the possible causes of the observed high positive selection pressure of these alleles, but there have to our knowledge as yet been no suggestions that they might confer protection against an increased pathogenic load since the rise of agriculture. Pathogens such as Mycobacterium tuberculosis, Rotavirus, E. coli, Mycoplasma pneumoniae and fungi producing highly toxic mycotoxins, together with gluten as a novel food ingredient, may cause devastating effects in humans through pathways ending up in dehydration, hyponatraemia and lack of energy, ending up in multiple organ failure (MOF). We suggest (our hypotheses) that improved lactose and starch digestion through lactase persistence and augmented AMY1-GCN provided a survival advantage by facilitating the activity of the immune system and the most important glucose, water and sodium transporter in the gut and thereby conferred protection against infections and infection-driven dehydration, hyponatremia and multiple organ failure.
1. Lactase persistence, existing hypotheses

1.1. The UVB-vitamin D-calcium-rickets hypothesis

Swallow et al. (9) related the higher and longer intake of milk and milk products to lactase persistence after infant age through the emergence of polymorphisms in the regulation of the lactase gene (11, 12). The hypothesis was subsequently supported by others (13). Several explanations have been given for the positive selection of these polymorphisms leading to lifelong lactase persistence. One of them highlights the advantage of the improved calcium intake if lactose could be digested (14). The low UV-B levels at high latitudes are associated with an increased risk of developing rickets and osteomalacia due to the lack of cutaneous vitamin D production during the long winter period. In the gut, the vitamin D hormone, 1,25-dihydroxyvitamin D, stimulates the expression of proteins involved in the absorption of calcium, which is itself an essential mineral required for bone health, signal transduction and others. Milk born calcium may also help to prevent rickets by impairing the breakdown of vitamin D in the liver (15).

Rickets narrow the female birth channel and thereby increases the chance of mortality of both mother and child during labour (16). The only successful preventive measure is by caesarean section, which has been part of human anthropology probably since millennia (17) and seems to have saved thousands of people also in recent times (16). However, Itan and Swallow (11), using a flexible demic computer simulation model to explore the spread of lactase persistence, challenged the vitamin D-rickets model by showing the absence of a relationship between lactase persistence and the requirement of more vitamin D in people living at Northern latitudes.

The vitamin D-calcium-rickets hypothesis based on the low ultraviolet-B (UVB) radiation at high latitudes does not hold in the light of the very low prevalence of lactase persistence among other individuals living in northern regions such as those living in Siberia or the Amerindians living in the north of America (18, 12). Lactase persistence reaches the highest prevalence in countries around the Baltic- and North Sea (19, 17), which coincides with extreme dermal depigmentation, unique for this part of the world population (20). It is widely accepted that depigmentation is a consequence of living at high latitude and lack of vitamin D producing UVB radiation. UVB radiation (280-340 nm) is necessary for the conversion of pre-vitamin D3 into vitamin D, which prevents the development of rickets. Pale skin obviously captures more sunlight because of the reduced absorption by melanocytes (21).

Although there is a trend for lighter skin colour with increasing latitude, no other population exhibits the extreme dermal depigmentation encountered in Europeans living in the circum Baltic/North Sea region, even not populations living at higher latitudes, which suggests an
additional geographic selection pressure. It is possible that populations in the North of Europe experienced another threat to their calcium homeostasis due to the spread of agriculture in general, and in particular because of the consumption of wheat and barley.

### 1.2. The influence of cereals

Cordain et al. (20) stated that ‘Whole cereals are rachitogenic because their high fibre content impairs the entero-hepatic circulation of vitamin D, leading to increased faecal elimination of 25-hydroxyvitamin D3, thereby progressively lowering plasma 25-hydroxyvitamin D3 concentrations in humans’. Moreover, whole grains are poor sources of calcium, and their high phytate content reduces calcium bioavailability and thereby additionally contributes to their rachitogenicity. The region around the Baltic/North Seas is one of the few high-latitude regions in the world where cereal grains can be successfully grown without intensive modern agricultural procedures. Usually, at high latitudes, extreme temperatures render the growing season too fleeting for cereal crops to effectively compete with animal foods as a staple. Because the warm Gulf Stream flows into the North and Baltic Seas and because of the nearness to a maritime heat sink, temperatures in the surrounding landscapes were sufficiently high to allow Neolithic farmers to successfully grow cereals as staples, primarily wheat and barley (20).

### 1.3. Our hypotheses: Lactase and the immune system

To our knowledge, there has been no suggestion of a relation between lactase persistence and the immune system in which lactase confers protection against potentially lethal pathogens. Apart from the digestion of lactose, lactase is important for the intestinal absorption of quercetin. By the hydrolysis of the naturally occurring quercetin-glucosides, quercetin absorption may increase drastically in the presence of lactase (22). Quercetin is one of the most abundant flavonols in edible plants (USDA database 2011) and is considered vital to maintain immune function (23). Epidemiological studies indicate that higher compared to lower quercetin intake is associated with reduced risk for ischemic heart disease, type 2 diabetes mellitus, asthma, and various types of cancer including lung cancer, colorectal cancer, prostate cancer (24) and has widespread antimicrobial effects against viruses, bacteria and fungi (25).

As stated in the introduction, pathogens have exerted strong selective pressure in human evolution (4). One of the most lethal infectious diseases in humans is and has been pneumonia (26). Every year 65,000 people in the USA die because of influenza and pneumonia (27). Pneumococcal load has been a strong selective pressure factor shaping the human immune system and genome (28). Another microbe producing pneumonia atypical in humans and pneumonia in cattle is Mycoplasma mycoides (29). Mycoplasma pneumoniae in
cattle and probably humans became important with the rise of domestication of cattle, including cows and goats, some 10,000 years ago (30). Domestication of cattle was the starting point of introducing milk and milk products into the human diet and also the time point when the polymorphism for lactase persistence typical for Europeans arose (12). Even now farmers seem to be at surprisingly high risk (10 to 50 times more than expected) for the development of pneumonitis (31).

Quercetin has effective antibiotic effects against bacteria producing pneumonia but only at high doses (32). These might be provided by lactase (32) suggesting benefits in immune function by lifelong lactase-facilitated quercetin absorption. We suggest that positive selection of lactase persistence is related with increased protection against potentially lethal pneumonia-causing pathogens.

1.4. Conclusions so far

Taken together, lactase persistence is a very recent polymorphism and its positive selective pressure has been extremely strong (33). It is hard to conceive that such a high selective pressure was caused by diseases with low lethality such as osteomalacia, and osteoporosis, or because of the adaptation to a novel food with nevertheless high caloric and micronutrient contents. On the other hand, rickets and pathogenic load are strong selective pressure factors. Pathogenic load has been the leading roller coaster of human evolution and it is therefore conceivable that subjects with lactase persistence are better protected against the mentioned pathogens. This hypothesis is easily testable by measuring the difference in infection susceptibility between lactase persistent and non-persistent individuals.
2. **Salivary AMY1 gene copy number**

2.1. **AMY1 copy number and starch consumption**

In 2007 Perry et al. (10) published a seminal paper relating the number of amylase (AMY1) gene copies to the amount of dietary starch. They found that the number of the salivary amylase gene copies correlates positively with salivary amylase protein levels and that individuals from populations with high-starch diets have, on the average, more AMY1 gene copies than those with traditionally low-starch diets. Since the publication of Perry et al., several hypotheses have been proposed to explain positive selection of augmented AMY1-GCN. More AMY1 copies and amylase protein could ‘buffer against the fitness-reducing effects of intestinal disease and toxicosis’ (10), although they did not specify what environmental factor(s) would cause intestinal disease.

2.2. **Existing hypotheses**

One hypothesis relates salivary amylase activity to satiety (34). This effect could buffer against overeating, although higher amylase levels during stressful situations may produce the opposite. For instance, emotional overeating in response to psychosocial stress is a behavioural trait related to childhood obesity and psychosocial stress factors increase salivary amylase production (35). Our society is characterized by food abundance, overeating increases the risk of obesity, and obesity is a risk factor for diabetes mellitus type 2, cardiovascular diseases (CVD) and others (36). A buffering effect against overeating could therefore decrease the incidence of CVD and related disorders. CVD and related disorders affect a wide range of people, CVD are still the major causes of mortality worldwide (37) and mortality is an important driving force in evolution by exerting selective pressure (38). It could therefore theoretically be possible that augmented AMY1-GCN developed through selective pressure of early mortality caused by increased obesity and CVD.

The above hypothesis, however, does not hold in the light of the estimated time of augmented AMY1-GCN occurrence and also not when considering that the capacity of overeating probably saved people from starvation at times when food was not available (39). The estimated time of appearance of augmented AMY1-GCN is around 200,000 years ago, although this estimate needs confirmation by the generation of AMY1 sequences from multiple humans (10). Obesity and CVD are widely considered modern diseases that arose very recently (40), while CVD mortality is highest among elderly people (41). At the time of the occurrence of the increase of AMY1-GCN, humans hardly reached an average age of 35 years, while even 200 years ago the average lifespan was only 30-40 years (42). It is therefore questionable whether obesity, CVD and mortality caused by overeating would have exerted the necessary high selective pressure on ancient populations in which the positive selection for augmented AMY1-GCN occurred.
A second hypothesis relates higher AMY1-GCN and increased amylase protein in saliva with certain sensory properties of starchy foods (43). Taste and viscosity could perhaps be detected earlier in individuals with higher AMY1-GCN, although the benefit of this trait is unclear (35, 43), making this hypothesis weak while lacking sufficient scientific strength to suggest positive selection.

Recently it has been shown by Mandel et al. (44) that, following the intake of starch-rich foods, individuals with high endogenous salivary amylase activity regulate glucose homeostasis better than individuals with lower salivary amylase activity (44). They state that efficient starch digestion could have had “immense benefits” and relate this benefit with protection against toxicosis and lower gastrointestinal malaise. This is exactly in line with our hypothesis which states that through more efficient starch digestion people were and are protected against multiple organ failure, dehydration and hyponatremia by upregulating the activity of SGLT1 (see our hypotheses).

It seems clear that no conceivable mono-hypothesis has as yet been generated for the augmentation of AMY1-GCN in human populations with high starch intakes. Adaptation to changes in food intake without evolutionary advantages, such as mortality before or during reproductive age or significantly reduced fitness, is unlikely to provide a sufficiently strong platform to explain accelerated positive selection. Before stressing our hypothesis, two other factors speak against the hypothesis that certain human populations have adapted to a nutrient, merely because it was incorporated in the diet.

2.3. **AMY1 expression relates to stress and pancreatic amylase activity is huge**

If humans adapted to starch rich food through increased expression of the amylase protein (related with higher AMY1-GCN), then why would amylase protein production be highest when salivary glands are (co)activated by the sympathetic nervous system (45)? It is widely recognized that the sympathetic nervous is the first wave of the acute stress responses. This would imply that starch intake produces stress, while stress is a reaction to danger (46). The logical consequence is that starch-rich food is dangerous: something living on starch-rich food is dangerous, or something within starch-rich food protects the starch-producing plant against something dangerous and the resulting compounds might be toxic to humans. The second factor that speaks against a merely nutrient intake driven genetic adaptation refers to the starch digestive activity of pancreatic amylase. As early as 1995 it was shown that pancreatic alpha-amylase extensively covers the digestive needs of starch intake (47). Pancreatic postprandial amylase enzyme output varies from 50 U/min to 2,000 U/min (69) with amylase having a digesting efficiency of 96% (48).
The significant difference in AMY1-GCN between populations with low and high starch intakes and between humans and our nearest evolutionary counterpart, the chimpanzee, is likely related to factors in or on starch-rich foods. High alpha-amylase facilitates breakdown of starch, liberating various monosaccharides, including glucose. The latter is present in roots and tubers eaten by the people belonging to the high AMY1-GCN tribes in Africa (49). Recent research with the Hadza, the last authentic hunter-gatherer population in Tanzania, shows that tubers and roots are an important part of daily food, varying from 18-38 en% of total caloric intake through the year (50). It is nevertheless their non-preferred food (50) and should be considered part of the fallback nutritional sources. Fallback nutritional sources normally provide energy when food is scarce. Given the importance of fallback food, it might be beneficial to optimize starch digestion efficiency. However, even if starch rich foods would exceed 38 en% of total intake, it would make no sense to increase salivary amylase level. Salivary and pancreatic amylase in combination with four other starch-digesting enzymes in the small gut would cover the starch digestion needs extensively (51).

2.4. **AMY1 and the immune system: domestic dog adaptations as introduction to our hypothesis**

It was recently found that domestic dogs exhibit several genetic changes suggesting adaptation to the intake of starch (52). The three genes showing intense selective pressure related with starch digestion in this study, are AMY2B, MGAM and SGLT1, which are responsible for the production of respectively amylase, maltase-glucoamylase and the sodium-dependent glucose co-transporter 1 (SGLT1). The difference in gene copy number, enzyme production and enzyme activity in dogs is highly significant compared with their most closely related counterpart, the wolf (52). The authors concluded that dogs have adapted to starch intake during domestication, suggesting that a change of ecological niche was the driving force behind that domestication and the novel niche could have induced scavenging behaviour in waste dumps. However, waste dumps do not only contain starch, but also other food wastes like meat, while all waste products come together with microbes, including Escherichia coli, Salmonella (both meat born microbes) and fungi such as Aspergillus growing on foul starch (53). These pathogens produce severe symptoms like vomiting, enterohemorrhagic enteritis, renal damage and failure, and death in humans, but especially among children, although adults are not spared (54).

The three genetic changes found in domesticated dogs and related with starch digestion, also have important effects on metabolism, systemic homeostasis and especially the immune system. We contend that the effects on the immune system have been the genuine background for the very high selective pressure on the above-mentioned genes in dogs and
that the same could hold for the AMY1-GCN in humans.

2.5. Evolutionary selective pressure aspects of the consumption of starch-rich foods; our hypotheses

2.5.1. Toxins in starch-rich foods
Starch-rich foods (SRF) contain, like most plants, a series of proteins and anti-nutrients with certain toxic effects. The mostly consumed SRF in non-African populations are cereals and legumes, whereas African tribes eat substantial amounts of tubers and roots, although cereals such as corn are rapidly replacing these ancient food sources (55). Whole grain cereals and legumes contain both toxic and non-toxic compounds and all of these substances contribute to the possible net toxic (nettox) effect of these SRF. It is important to consider only constituents present in whole grains as possible selective factor, because refined cereal products entered our diet only very recently. Candidate substances are gluten and its main component gliadin, certain digestive enzyme inhibitors such as amylase-inhibitors in wheat and lectins. A review was recently published on the potentially toxic impact of grains on human health (56).

2.5.2. Did gluten-toxicity exert sufficient selective pressure to cause augmented AMY1-GCN?
Gluten can cause a wide range of disorders, varying from celiac disease (57), autoimmune disorders (58), increased intestinal permeability syndrome (59) and types 1 and 2 diabetes mellitus (60) and gluten burden is not restricted to gluten intolerant individuals (61).

All of these disorders usually evolve with gastro-intestinal problems and often diarrhoea (62). The majority of mentioned disorders are negatively correlated with fertility and reproductive success (63). Diarrhoea can cause severe dehydration and sodium deficiency and both lead to increased mortality in both genders and at any age, but they affect children the most. Thus, at least theoretically, it seems possible that gluten intake affected reproduction and mortality by dehydration and severe sodium deficiency, causing selective pressure on cereal-eating populations. Improved digestion of starch might increase glucose levels in the gut, facilitating water, sodium and glucose transport by the sodium-dependent-glucose co transporter (SGLT1) and thereby protect against lethal dehydration, hyponatraemia and multiple organ failure because of energy deficiency, supporting our basic hypothesis and knowing that higher glucose levels in the gut activate SGLT1, whereas normal levels do not (64).

Identification of gluten as a trigger of severe gastro-intestinal complaints, including uncontrollable diarrhoea, vomiting and abdominal pain, occurred after World War II, when the Dutch paediatrician Willem-Karel Dicke noticed that the war-related shortage of bread in The Netherlands caused a significant drop in death rate among children affected by celiac disease—from greater than 35% to essentially zero. He also reported that once wheat was again
available after the war, mortality soared to previous levels. Following up on Dicke’s observation, other scientists looked at the different components of wheat, discovering that the major protein in that grain, gluten, was the culprit (65).

Celiac disease is the best-known disorder related with gluten intake and is caused by a combined impact of environmental factors on perhaps more than one hundred genes (66). Several genes seem to play major roles in subjects who are susceptible to celiac disease. They include genes for the production of human lymphocyte antigen (HLA) molecules and non-HLA genes for the production of interleukins and their receptors (66). The whole group of genes relates to the immune system and therefore it could well be that these genes originally protected against pathogens and that celiac disease only occurred after the incorporation of cereals into the human diet. In this direction, it has recently been shown that several interleukin/interleukin receptor genes involved in the pathogenesis of celiac disease have been subjected to pathogen-driven selective pressure. Specifically, celiac disease alleles of IL18RAP, IL18R1, IL23, IL18R1 and the intergenic region between IL2 and IL21 display higher frequencies in populations exposed to high microbial/viral loads, suggesting that these variants play a role in the response to these organisms (67). People with these genotypes are probably better protected against pathogens, but at the expense of celiac and other autoimmune diseases.

Gluten intolerance further produces secondary lactose intolerance, because of its damaging effect on enterocytes, which form the outermost layer of the gut (68). The combined effects of damage inflicted by the immunological response against gluten and the secondary lactose intolerance only increases the possibility of developing severe intestinal trouble including the typical lactose malabsorption symptoms, abdominal pain, diarrhoea, nausea, bloating, and/or flatulence (69).

The only plausible explanation for the positive selection or the preservation of genes that confer higher susceptibility to toxic-gluten, has to be related with something even more dangerous. People living in a highly infectious environment maintained or developed the need for increased immune reactivity against pathogens (4). When they incorporated gluten rich foods into their diets, the consequences of diarrhoea and vomiting could and undoubtedly have killed a countless number of people, demanding another phenotypical/genotypical adaptation to survive this novel selective pressure factor and this risk only increases when facing secondary lactose intolerance. And for this, why not employ another nutrient present in the same food to overcome the deleterious effect of the toxic part of this altogether important energy-providing nutritional source? Increased breakdown of starch in gluten-sensitive people would provide sufficient glucose to activate SGLT1 and
facilitate glucose, sodium and water transport and thereby prevent the severe burden of gluten intake. Increased amylase production through higher AMY1-GCN could serve this purpose.

The previously mentioned study of Axelsson et al. (52) shows that domesticated dogs carry an increased number of pancreatic amylase genes (AMY2B) and a special SGLT1 haplotype with structural (better function but not more enzyme) benefits compared with wolves. Dogs suffer from food allergies, just as humans, with almost identical symptomatology, including gastroenteritis-caused vomiting and diarrhoea, while canine gastroenteritis seems related with intestinal bacterial overgrowth with E. coli as main pathogen (70) and gluten intolerance. It has even been shown that gliadin, the main protein in gluten, produces the most severe allergic response in dogs (71), while dogs are used to investigate the pathways leading to clinical celiac disease because of the spontaneous development of celiac disease when fed with gluten-rich foods (72). This provides evidence for a parallel evolution of humans and domesticated dogs through increased starch digestion by amylase and improved glucose transport, protecting the host against possible toxicosis by a novel nutrient, such as gluten and/or certain pathogens, such as enterotoxin E. coli. Higher AMY1-GCN and amylase expression can serve this purpose perfectly and it were both Perry et al. (10) in their first publication on augmented AMY1-GCN and Mandel et al. in 2012 (44) who already pointed at the possible beneficial effects of augmented AMY1-GCN for the protection against intestinal toxicity.

2.5.3. Can starch itself be sufficiently toxic to produce important selective pressure?
Starch is the storable form of energy produced by all green plants.
A part of dietary starch escapes digestion by all enzymes, reaching the colon as resistant starch, ranging from approximately 1% following the consumption of white rice and 6% after eating beans (73). Incompletely digested starch has significant impact on the gut microflora in cattle and humans. Cows fed cereals can have a 1,000 times higher level of Escherichia coli in their gut and gut E. coli corresponds with higher detectable E. coli levels in their meat (74). Escherichia coli O157:H7, one of its most toxic mutants, can live undetected in the gut of food animals and can be spread to humans directly and indirectly. E. coli is a normal inhabitant of the gastrointestinal tract of mammals. Most E. coli strains do not cause disease, but can release lipopolysaccharide complexes from their cell walls (including lipid A) upon disintegration. These endotoxins can cause fever, and even death, but mostly if E. coli translocates from the gut into the blood. Traditional models of E. coli pathogenesis were based on the ability of certain strains to attach to mucosal surfaces, but the invasion process itself was poorly understood.
Cattle born pathogens are considered zoonoses (transferable to humans) and they include E. coli, but also Salmonella, Campylobacter and Clostridium difficile (75). Salmonella can be highly toxic as evidenced by various severe Salmonella outbreaks through ancient and recent history (76). Salmonella grows, just like E. coli on meat, and Salmonella/E. Coli infected meat and poultry are still considered the most important sources of food born illness (76). Survival of the pathogenic form of these microbes is facilitated by the intake of resistant starch that escapes total digestion in cattle (74). Recent research shows that this also holds for humans and that pathogenic growth of gut damaging microbes is substantially enhanced by maltodextrin, a derivate of incomplete digested starch (77).

It seems obvious that the combination of direct exposure to food born pathogens (E. Coli, Salmonella) and the nourishing effect of digestion-resistant starch has benefitted the growth of these microbes and thereby caused severe gastro-intestinal disorders in humans consuming infected meat and starch rich food. Macronutrient uptake is greatly dependent on gut surface, passage time and hydrolysis. Complete digestion of starch could have prevented bacterial overgrowth of pathogenic bacteria, offered protection against possibly severe gastroenteritis and even death. Augmented AMY1-GCN could have served this purpose, by causing a more rapid and thereby more complete digestion and uptake in combination with the other starch-digesting sacharidases.

2.5.4. Fungi growing on starch rich food; a largely ignored global health issue at present and definitely in the past

Starch provides the major food source for a wide range of fungi, including Fusarium species, Aspergillus flavus, Penicillium viridicatum and Acremonium coenophialum (78), whereas another fungus, Claviceps purpurea, parasites on starch rich cereals such as barley, rye and wheat (79). All of these fungi produce various types of highly toxic mycotoxins, including aflatoxins, tricothecenes, fumonisins, T-2 toxin, zearalenone, deoxynivalenol and ochratocin A (80). Among these, Claviceps purpurea is a special fungus, because it has historically often been looked at as a part of the cereal plant although it produces the most severe mycotoxins named ergopeptines, including ergometrine (81).

When present in foods in sufficiently high quantities, mycotoxins can produce symptoms ranging from acute liver or kidney deterioration, severe gastroenteritis, vomiting, anorexia, reduced weight gain, neuroendocrine changes, immunological effects, diarrhoea, leukocytosis, haemorrhage or circulatory shock, and acute death, to chronic liver cancer, mutagenic, and teratogenic effects, skin irritation, immunosuppression, birth defects, neurotoxicity, and ‘slow’ death (82, 83).
Acute mycotoxicosis belongs to the most devastating infections, affecting human beings as evidenced by several recent outbreaks including the one in Kenya in 2004, that affected 317 individuals, killed 125, and was caused by the ingestion of aflatoxin-infected maize (84). Another fatal outbreak in 1974 killed 100 persons in India because of the consumption of aflatoxin-infected corn (83). Not only cereals are a rich source of mycotoxins. Other starch rich foods such as dried fruits (83), beans, peanuts (83) and underground bulbs (84) are also frequently affected by fungi, producing different types and amounts of mycotoxins. The actual economic burden of affected crops is enormous, because of the difficulties to control fungal growth on SRF (85) and the estimated economic losses are $1.4 billion every year only in the USA (83). In the European Union, regulations limit the amount of total aflatoxins to 4 ng/g, whereas guidelines in a few developing countries and the US limit total aflatoxins to no more than 20 ng/g in foodstuffs intended for human consumption (86). In Nigeria, the National Agency for Food, Drug Administration and Control has set 20 ng/g as the maximum permissible limit for total aflatoxin in foodstuff (86).

Even though preventive methods are very well defined, mycotoxins keep affecting 25% of world crops (87). When people started eating SRF thousands of years ago, logical reasoning informs us that those crops must have been affected by fungal infections and that the consumers are likely to have suffered from acute and/or chronic mycotoxicosis. The group of Lesley investigated how mycotoxin growth and fatal doses of mycotoxins could be prevented in non-industrialized countries, which were basically all countries of the world 10-30,000 years ago (88).

These methods are nowadays likely to be well known in the majority of SRF eating populations, but it is unlikely that ancient populations knew how to deal with fungal growth on SRF, while even if they did, it does not imply that they avoided consumption of the affected foods. Even today situations of relative scarcity of food often forces consumers in many regions to distressing decisions, such as ‘to eat contaminated grain today and worry about the consequences tomorrow (or some other time in the future) or starve today and perhaps not even have a tomorrow’ as stated by Bandyopadhyay (89). This behaviour is not a unique ‘characteristic’ of modern humans, it is the way most individuals think and have thought when starving to death.

Several data suggest that people have suffered from more or less fatal mycotoxicosis for hundreds of generations and mycotoxin intoxication has without doubt killed thousands of people. The mortality rate ranges from 10-60% of the infected people and embryos and children are among the most affected individuals (90). Important proof for the devastating effects of mycotoxins comes from data related with ergotism. Ergotamine and other
ergopeptines from the fungus Claviceps purpurea are highly toxic to humans and can cause different types of ‘ergotism’. Convulsive, gangrenous and entero/hyperthermic ergotism cause respectively epileptic convulsions, confusion and death (convulsive), vasoconstriction, cold/hot feeling, abortion and gangrene (gangrenous) and nausea, vomiting, diarrhoea, increased metabolic rate and excessive salivation (entero/hyperthermic, 80). Convincing historical data for the existence of epidemics of mycotoxicosis are as old as 600 BC, when people in Assyria suffered from ‘madness’ caused by ‘the noxious pustule in the ear of grain’ (91) and this seems to be the same disease that affected many parts of Europe in the tenth century referred to as St. Anthony’s or Holy fire, and is considered to have been caused by the consumption of rye contaminated with ergot alkaloids from Claviceps purpurea. Further accounts suggest that other contaminated grains have been responsible for major outbreaks of disease (e.g., the Ten Plagues of Egypt, 83).

Mycotoxins can produce chronic diarrhoea and vomiting. At the same time they highly influence the functionality of SGLT1 and GLUTs. Very low doses of mycotoxins inhibit nutrient transport in the gut, especially affecting SGLT1 (50% inhibition with a 10 µmol/L solution in vitro) and the fructose transporter GLUT5 (42% inhibition, 92). Later studies in animals confirmed these findings (93). Mycotoxins produced by Aspergillus further inhibit alpha-amylase activity, which has been evidenced several times in vivo, although mostly in chickens (94). The combination of these targets and their effects can be considered the perfect cocktail to die because of dehydration, hyponatraemia and MOF.

Aspergillus and its toxins are definitely highly toxic and have killed thousands of individuals, including children and adolescents (95).

The described pathways, by which mycotoxins can have affected human health and even mortality rate, could, and probably have, served as important selective pressure factors for the observed augmentation of oral AMY1-GCN in populations with high starch intakes. Complete starch digestion would have provided enough glucose to augment SGLT1 activity, improving the transport of glucose, water and sodium, preventing dehydration, multiple organ failure and possibly lethal hyponatraemia in infected individuals.

Of all the pathogens described in this paper and considered as possible cause of selective pressure on the number of AMY1 gene copies, fungi producing mycotoxins seem to be the most appropriate candidates.

2.6. Conclusions so far

The entrance of SRF in the human diet introduced several positive and negative factors. Gluten in SRF can be highly toxic and the incomplete digestion of resistant starch in SRF
provides an optimal substrate for the growth of possibly lethal pathogens including food borne toxic E. coli strains and Salmonella. Dehydration, hyponatraemia and MOF are the most damaging effect of gluten-toxicity and food borne pathogens due to chronic diarrhoea and vomiting. The same holds for fungi living on SRF, producing highly toxic mycotoxins that can be fatal. Higher salivary alpha-amylase activity through augented AMY1-GCN can increase glucose level in the gut, facilitating water, sodium and glucose uptake and protect against the above mentioned effects. The observation that people with higher AMY1-GCN have lower blood glucose levels after starch intake supports the latter conclusion, suggesting that this polymorphism has been positively selected for higher glucose level in the gut.
3. **Rotavirus, lactose intolerance and AMY1-GCN**

Rotavirus is one of the most important causes of gastro-enteritis, malnutrition, and diarrhoea in young children and animals. Rotavirus can be extremely dangerous, evidenced by the fact that more than 550,000 children die every year from this infectious disease (96). Rotavirus induces diarrhoea and rotavirus-diarrhoea can be lethal because of reduced uptake of sodium and subsequent systemic hyponatraemia through a direct inhibiting effect of rotavirus on the SGLT1 mechanism (97). Rotavirus further induces lactose intolerance by inhibiting lactase production (98) and dairy intake in people suffering from rotavirus diarrhoea seems to augment the diarrhoea duration (99). As mentioned before, rotavirus is extremely dangerous for children and newborn and is the major death cause in children younger than 5 years old (96). Newborns produce lactase to break down the lactose in their mother’s own milk, converting it into glucose and galactose, providing energy and enough glucose to transport water, sodium and glucose through activation of SGLT1. Newborns hardly produce endogenous amylase up to 6 months and even after 2 years still show some dependency on breast-milk amylase to support normal starch breakdown (100). Nothing in breast-milk needs amylase to become digested, so why would breast-milk contain substantial amylase activity?

It has been proposed that breast-milk amylase could serve as a compensation for low salivary and pancreatic amylase activities in newborns and aid in the digestion of complex carbohydrates from the time that complementary foods are introduced in close proximity to breastfeeding (101). We suggest that the substantial amylase level in breast-milk has become needed to digest complex starch rich food, at times that pathogens, but especially rotavirus, cause diarrhea through their combined disturbance of lactase activity, damage to enterocytes and inhibition of sodium transport (102).

Rotavirus might be considered an important selective pressure factor (103). It has probably entered the human environment as a zoonose from domestic animals (104). The majority of individuals dying from rotavirus infections live in developing countries; countries where domestic animals and novel zoonoses are relatively new. We suggest that augmented AMY1-GCN has been selected for the protection against lactose intolerance in the newborn caused by pathogens in general but more specifically by Rotavirus. High amylase level in breast-milk facilitates starch digesting in newborns that suffer from Rotavirus-induced lactose-intolerance. Improved starch break down increases glucose level in the newborn gut, activates SGLT1 and guarantees a minimum of sodium, water and glucose transport into the bloodstream and thereby protects against possibly lethal hyponatraemia, dehydration and MOF. This is exactly the rationale behind oral rehydration in individuals suffering from Rotavirus diarrhoea (105). Rotavirus is nowadays hardly fatal in North European countries. Augmentation of AMY1-GCN could be at the basis of the lack of virulence of Rotavirus in this
part of the world, whereas Rotavirus in Africa is still a major cause of childhood and overall mortality (99, 105).
4. Discussion of our hypotheses and final conclusions

Table 1 shows an overview of the different environmental factors that might have caused selection for lactase persistence and augmented AMY1-GCN. We suggest that these factors are not mutually exclusive, but should be viewed upon as complementary and logical in the scene of evolutionary biology. Lactase persistence and augmented AMY1-GCN are two genetic adaptations showing intense positive selection (lactase persistence > AMY1-GCN) in humans. Lactose and starch have deleterious effects on human health when digesting is incomplete and this occurs when lactase and amylase do not reach sufficiently high activities. Lactose intolerance (not treated in this review) itself can be detrimental to human health, through causing symptoms like irritable bowel syndrome, watery stool and excessive flatus (106). Lactose intolerance can nevertheless hardly have served as the only factor in the observed highly positive selection of lactase persistence, because of the lack of sufficient influence on mortality (107), while lactose intolerance also does not seem to affect fertility (108). A recent publication (109) also challenges the rather simple ‘fresh milk intake’ hypothesis as the driving force behind lactase persistence.

Incomplete starch digestion also affects human health, but without bacterial overgrowth of for instance Escherichia coli, symptoms would be troubling, but mortality rate and reproduction are less affected. Looking at both genetic adaptations in a broader perspective suggests that they might protect against the severe deleterious effects of several pathogens and the damaging effects of gluten. Pathogens like Rotavirus affect lactose digestion directly, whereas incomplete starch digestion facilitates the growth of possibly lethal cholera like E. Coli strains. Humans have explored new environments more than any other animal and novel environments challenge the immune system with new climatological conditions, food and, most of all, pathogens.

The last big revolution in the human evolutionary history has been the development of agriculture: somewhat like 10 thousand years ago someone started to exploit the observation that a plant growths from a seed and agriculture started. With agriculture came the domestication of cattle and with cattle came new pathogens. The evidence we have given in this article supports our hypotheses that it are these factors that have driven the highly positive selection of lactase persistence and augmented AMY1-GCN: both adaptations provide enough glucose to activate SGLT1, which is the most important glucose, sodium and water transporter in the gastrointestinal tract. The increased capacity of water, sodium and glucose transport from the gut into the blood stream, has probably saved thousand of individuals infected with pathogenic E. Coli, Rotavirus and TB, but most of all from highly lethal fungi living on starch rich food. The adaptations also protected them against the highly damaging effects of gluten intake. We therefore contend that lactase persistence and augmented AMY-
GCN are not merely adaptations to nutrients in novel foods; they were needed to protect humans against novel pathogens that arose with agriculture and the domestication of cattle. Food born pathogens, living on meat and starch rich foods are still serious threats to human health and it is therefore necessary to further investigate how we can limit the presence of possibly lethal pathogens in human and animal foods.
**Table 1. Overview of the proposed mechanisms for the strong positive selection of lactase persistence and augmented AMY1-GCN.**

<table>
<thead>
<tr>
<th>Selective pressure factor</th>
<th>Evolutionary target</th>
<th>Consequences</th>
<th>Contemporary solution</th>
<th>Evolutionary adaptation</th>
<th>Benefit</th>
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<tbody>
<tr>
<td><strong>Milk</strong></td>
<td>Lactose intolerance</td>
<td>Diarrhoea, Rickets. Energy and calcium deficiency,</td>
<td>Lactose free dairy products Vitamin D Calcium</td>
<td>Lactase persistence</td>
<td>Availability of important macro- and micronutrients including vitamin D and Calcium</td>
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<tr>
<td><strong>Starch</strong></td>
<td>Incomplete digestion</td>
<td>Intestinal malaise. Energy deficiency</td>
<td>Higher salivary amylase production and activity by higher AMY1-GCN</td>
<td></td>
<td>Availability of important macro- and micronutrients</td>
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<tr>
<td><strong>Mycoplasma Pneumonia (MP)</strong></td>
<td>Immune system</td>
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<tr>
<td><strong>Gluten in SRF</strong></td>
<td>Gut barrier SGLT1</td>
<td>IIPS, chronic diarrhoea, vomiting, loss of fertility, death</td>
<td>Gluten free cereals</td>
<td>Higher salivary amylase production and activity by higher AMY1-GCN</td>
<td>Higher gut glucose and activation of SGLT1. Protection against dehydration, hyponatraemia and MOF</td>
</tr>
<tr>
<td><strong>Starch</strong></td>
<td>Incomplete digestion</td>
<td>E. coli overgrowth syndrome. Diarrhoea, bladder infection</td>
<td>Antibiotics Oral rehydration</td>
<td>Higher salivary amylase production and activity by higher AMY1-GCN</td>
<td>Higher gut glucose, activation of SGLT1. Protection against dehydration, hyponatraemia and MOF. Availability of mannose; effective against E. coli infections</td>
</tr>
<tr>
<td><strong>Agriculture and food born pathogens (zoonoses), especially Fungi. but also E. Coli, Salmonella, Campylobacter, Clostridium</strong></td>
<td>Immune system SGLT1</td>
<td>Mycotoxins. Diarrhoea, vomiting, gangrene, abortion, epilepsy, infant death by DH, HN and/or MOF</td>
<td>Antibiotics Oral rehydration Antifungal treatment</td>
<td>Higher salivary amylase production and activity by higher AMY1-GCN</td>
<td>Higher gut glucose, activation of SGLT1. Protection against dehydration, hyponatraemia and MOF.</td>
</tr>
<tr>
<td><strong>Rotavirus</strong></td>
<td>Lactase gene Immune system</td>
<td>Lactose intolerance, lethal diarrhoea mostly in newborn</td>
<td>Oral rehydration Alternative feeding</td>
<td>Higher salivary amylase production and activity by higher AMY1-GCN</td>
<td>Increased amylase in breast milk, improving digestion of SRF when the newborn developed lactose intolerance and needed complex food to survive</td>
</tr>
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References


