

Title:

**Agrarian diet and diseases of affluence
- Do evolutionary novel dietary lectins cause
leptin resistance?**

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ABSTRACT

The global pattern of varying prevalence of diseases of affluence, such as obesity, cardiovascular disease and diabetes, suggests that some environmental factor among agrarian societies could initiate these diseases. The environmental factor in question might be an evolutionary novel agrarian diet containing large amounts of seeds from grass. Leptin resistance is an acquired insensitivity to high levels of the satiety factor leptin associated with diseases of affluence. We conclude from recent studies on the molecular evolution of leptin that human leptin is not specifically adapted to an agrarian diet, but that such a specific adaptation of leptin through convergent evolution possibly accounts for the high similarity of leptin genes between birds and rodents. It follows that human leptin resistance could be due to insufficient adaptation to an agrarian diet. Cereal lectins might be the constituent of an agrarian diet causing leptin resistance either indirectly, through effects on metabolism central to the proper function of the leptin system, or directly, through binding to human leptin or leptin receptor and affecting their function. If dietary lectins could negatively affect leptin binding affinity this should be lower in susceptible humans on an agrarian diet which is supported in studies on the effects of fasting on obese and lean subjects.

BACKGROUND

Thomas McKeown suggested a classification of diseases according to their causation, where all diseases which one may contract after birth could be divided into diseases of poverty and diseases of affluence [1]. The diseases of affluence included the so called Western diseases, which are related to a western lifestyle and characteristic of more economically developed and affluent countries [2, 3]. There is a global variation in the prevalence of diseases of affluence, such as obesity, cardiovascular

disease and diabetes. When looking at this variation it is possible to see different patterns depending on your particular point of reference. In this paper our point of reference will be types of societies regarding their way of acquiring food. We will thus make a division between agrarian and non-agrarian societies. Non-agrarian societies can be further divided into hunter-gatherer and horticultural societies. Hunter-gatherer societies acquire the major part of their food from hunting, fishing and gathering wild plants and insects, and the components of a hunter-gatherer diet therefore have varied due to local conditions. A hunter-gatherer diet is characterized by great variation of food sources, high levels of nutrients (vitamins, minerals, trace elements) and absence of cereals, dairy products, refined fat and sugar. In many cases cultivated crops supplement the diet but they provide only a minor part of it. Hunting and gathering is thought to represent the original mode of life common to all prehistoric humans during the Paleolithic (i.e. the Old Stone Age 2.6 million-10,000 years ago) [4, 5]. Horticultural societies obtain the bulk of their food from gardening. This sometimes implies heavy dependence on a single starchy cultivar such as a root crop (e.g. manioc).

The diet in an agrarian society is based on agricultural products from tending livestock and fields of grass. An agrarian diet thus includes a large amount of seeds from grass commonly referred to as cereals (e.g. wheat, rice, maize). Cereals are per definition rare or absent in the diet of non-agrarian societies. The special cases of agrarian societies which only tend livestock and not fields (pastoral people e.g. the Masai of Africa) will not be discussed in this paper, although they seem to conform to the disease pattern described below [2].

DISCUSSION

Also among agrarian societies there is a varying prevalence of diseases of affluence. The variation is exemplified by Japan where the mortality from diseases of the circulatory system, such as stroke and ischemic heart disease, as compared to other causes of death is 34 %. This is lower than

in the United States or Sweden (41 % and 49 % respectively) [6]. However, all these numbers are still high when compared to non-agrarian societies such as the horticultural Trobriand islanders with a mortality from diseases of the circulatory system which apparently is close to zero even though they have access to abundant sources of food, smoke heavily and have a fair share of elderly people (the oldest person, a woman, had an estimated age of 96 years) [2, 7]. These disproportionate numbers between agrarian and non-agrarian societies differ even more when considering only the incidence of non-infectious stroke [8, 9]. A natural objection to such comparisons would be that the Trobriand Islanders and people living in other non-agrarian societies are somehow genetically protected from these diseases. However, people living in non-agrarian societies seem to contract these diseases of affluence upon migration to an agrarian society or when their own society becomes one [2, 7].

Examples of non-agrarian societies in transition towards or with recent change into an agrarian society are the Amazon-dwelling Brazilian Indian tribes Amondava and Parkateje. The Amondava is described as one of the most isolated populations in the world but still has a diet which incorporates the cereals rice and maize. The Amondava has no overweight, obesity or diabetes [10], and could be taken as an example of a society which incorporates agrarian products without any obvious harm with regard to the diseases of affluence. The reasons behind the apparent absence of diseases of affluence among the Amondava could be many. Genetics is of course one, but that would seem to be an exception to the rule [2]. It could be a matter of timing, such as a recent shift in diet with insufficient time to develop disease. Such a pattern of differentially delayed onset of the various diseases of affluence has been described [2]. It could also be a matter of degree, such as when the incorporated agrarian products only contribute a minor part in an overall horticultural diet. This is possibly the case of the Amondava, since their diet is described as being rich in dietary fibres and complex carbohydrates with the main components being rice, beans, maize, manioc, potatoes and wild

vegetables [10]. The Amondava illustrate that the cause behind the initiation and progression of diseases of affluence are most certainly multi-factorial and probably several factors in sufficient degree need to be present for these diseases to appear clinically.

The other Indian tribe Parkateje is described as having suffered rapid and intensive cultural and dietary changes in recent years, and has a very high prevalence of 82.2 % of overweight or obesity and a prevalence of diabetes among adults of 3.3 % [11]. The Parkateje illustrate that there is no genetic protection against diseases of affluence among this population that was until recently non-agrarian. The high prevalence of overweight among the Parkateje also illustrate the 'overkill' effects of rapid and severe acculturation when the incidence and severity of the diseases of affluence rises rapidly until it may exceed the levels found in societies which made the change over the course of a long period of time [2].

The global pattern of varying prevalence of diseases of affluence thus suggests that some environmental factor among agrarian societies could initiate these diseases. In this paper we shall look closer at cereals which set an agrarian diet apart from a non-agrarian. Earlier works have explored some of the potential nutritional problems with cereals such as vitamin deficiencies and content of antinutrients [12]. Since nothing in biology makes sense except in the light of evolution [13], we shall take a look at human diet from an evolutionary perspective.

Human diet and evolution

The human species belong to the comparatively small mammalian order *Primates*, which emerged between 90 and 65 million years ago (mya) [14, 15]. The last common ancestor of living primates is thought to have been a mouse-sized animal hunting insects which took to the trees in search of food [14, 15]. The grasses, which all belong to the family *Poaceae*, evolved between 65 and 55 mya [16]. Since the last common ancestor of living primates emerged before this time, it cannot have had a diet consisting of seeds from grass. Subsequent primate evolution is thought

to have taken place in the trees where almost all potential plant food comes from dicotyledonous species [17]. Dicotyledonous (in modern systematics replaced by eudicots and magnoliids [16]) and monocotyledonous plants make up the two suborders of the flowering plants, which is the largest and most diverse group of plants today. Among the monocotyledonous species not available in the tropical forest to the ancestral line of primates are the grasses. Our primate ancestors probably remained well adapted to an arboreal way of life and diet up until 8-4 mya [18-21]. From this time onwards our ancestors became more terrestrial and associated with mixed to open environments, such as savannah with grass. Unfortunately, the archaeological evidence of the diet of our ancestors during the last four million years of evolution towards modern humans is weak. The best interpretation of the available data seems to be that our ancestors have been very successful as highly adaptable omnivores, who probably had a significant input of animal products into their diets [22]. If grass seeds were being incorporated into the diet of our ancestors, they probably only contributed a small part. Our own human species of *Homo sapiens* is believed to be only about 200,000 years old [23, 24]. The conclusion regarding *Homo sapiens* hunter-gatherers seems to be that they could well have eaten seeds from grass, but since they do not appear to have gone to any great length to process them, they were probably not key dietary staples [25].

This means that the global human population is probably not fully genetically adapted to a diet with large components of grass since they do not share a common ancestor that has ever encountered such a diet. It is not until about 20,000 years ago the archaeological records begin to show tools for processing plants and possibly cereals, and 10,000 years ago some humans definitely encountered this diet when they started practising agriculture [25]. From an evolutionary perspective, this means a great shift in subsistence base merely 500 generations ago. However, the transition from the hunting-gathering mode of life to agriculture took place at different times in different places of the world. For many people

this shift to a diet with large components of grass happened more recently, between 1-100 generations ago. This is in an evolutionary perspective a very short time, especially when considering the diseases of affluence whose evolutionary pressure would affect mainly older people after their reproductive period and thus only could exert a lesser influence on individual fitness [26]. Some populations which have practised agriculture for several thousand years possibly have some genetic adaptation to an agricultural diet such as lower prevalence of celiac disease and related HLA genotypes [27], but apparently not enough to protect them from diseases of affluence. Thus, when examining human diet from an evolutionary perspective it makes sense that humans with an evolutionary novel agrarian diet could suffer from diseases due to insufficient adaptation. We suggest that leptin resistance could be a sign of such insufficient adaptation.

Leptin resistance

The satiety factor leptin is a common denominator of the diseases of affluence and their associated disturbed metabolism. Leptin (from the Greek root *leptos* meaning thin) is a protein mainly synthesized in white fat tissue in proportion to the amount of stored body fat. It is also synthesized in other tissues of the body such as brown fat, the placenta, stomach, mammary epithelium, pituitary, hair follicles and bone. Leptin acts as a signal to the brain to inhibit food intake (hence the denomination satiety factor) and also has peripheral effects on such diverse systems as the regulation of the growth of blood vessels, the immune system, the reproductive system, glucose- and fat metabolism and bone growth [28, 29]. In a recent review our current understanding of the leptin system seems to agree with evolutionary theory [30]. Apart from regulating appetite, leptin thus enables the storage in adipocytes of the surplus calories required to survive famine while simultaneously protecting peripheral non-adipose tissue from toxic effects of intracellular lipid overload [30].

The concept of leptin as a satiety factor gave rise to early hopes of successful treatment of obesity, but administration of leptin in humans did not give expected positive results in the form of significant weight loss seen in experimental animals such as rodents [29]. The great majority of obese humans have high levels of leptin correlating to their body fat mass, suggesting that insensitivity to leptin rather than lack of leptin might be the cause of their obesity [29, 31]. This insensitivity to leptin is termed leptin resistance, which is an acquired insensitivity to high levels of leptin associated with diseases of affluence [29, 32-37]. Some forms of end-organ resistance can be caused by mutations in hormone receptors, which have been described for most classes of hormones, but the pathophysiology of acquired forms of end-organ resistance such as insulin and leptin resistance has been elusive [38].

The differing results from leptin administration implies that the detailed actions of leptin in energy metabolism are different in humans versus experimental animals such as rodents [39]. This difference could be genetically based and possibly an adaptation to some environmental factor, which through selection has changed the leptin system of either humans or experimental animals. But this difference could also be due to insufficient adaptation to some environmental factor, which is now affecting the leptin system of humans or experimental animals. To address these different possibilities we turn to recent studies on the molecular evolution of leptin. In order to fully understand the implications from these studies we first explain the concept of molecular evolution.

Molecular evolution

Leptin has been found in many mammals and also among birds [40], which taken together with its wide and diverse distribution of production sites in the body and also variety of function and effect [28, 29] is suggesting an ancient evolutionary history [41]. Like all genes and their transcribed proteins, leptin is subject to change due to molecular evolution. This molecular evolution is caused by random genetic events,

which change nucleotides (or bases) in the DNA. Evolution at the molecular level is hence observable as nucleotide (or base) changes in the DNA [42]. Some nucleotide changes are termed *synonymous* (SYN) since they do not alter the transcribed amino acid. This is because the nucleotides are transcribed in triplets, and some of these different triplets are transcribed into the same amino acid. That means that a SYN nucleotide change in a gene will not alter its transcribed protein. A SYN nucleotide change will therefore not affect individual fitness and cannot be affected by natural selection. Its presence and frequency in a population is therefore dependent upon random events entirely. As opposed to SYN changes, a *non-synonymous* (NS) nucleotide change alters the transcribed protein, and can therefore be affected by natural selection. NS changes with negative effect on individual fitness will get sorted out by natural selection and will not spread in the population. NS changes with no net negative or positive effect on individual fitness cannot be sorted out by natural selection and are termed neutral. NS changes with positive effects on individual fitness are termed positive, since they are favoured by natural selection and will spread in a population. Such genetic change through natural selection constitutes an adaptation. Thus, if DNA was preserved from dead animals and extinct species, we could closely follow the genetic changes through time and infer when and where adaptation took place. But DNA is not preserved like bone or teeth and does not fossilize. Instead, molecular evolution is studied indirectly by comparing genes from animals in extant (living) species.

Molecular evolution of human leptin

Humans are placed in the superfamily Hominoidea, which is a group of higher primates consisting of gibbon, orang-utan, gorilla, chimpanzee, early human and modern human. The hominoids emerged as a group distinct from other primates some 25-30 mya and was successful in penetrating the tropical arboreal plant food niche with a special emphasis on fruits [15]. Studies on leptin have shown a significant increase of NS to

SYN changes in the ancestral line of primates giving rise to hominoids, and this significant increase is relative to all other mammals and to its immediate primate ancestor and to its descendant hominoids including extant species such as humans [39]. This means that the ancestral line of primates giving rise to hominoids probably acquired several positive NS changes of their leptin gene due to adaptations. It also tells us that the leptin genes of humans have not changed much since the time of the primate ancestor common to all hominoids. Thus, it is very unlikely that human leptin could be specifically adapted to an agrarian diet containing large amounts of seeds from grass that humans have encountered only recently. But this is not the same as saying that such a diet must be a problem to the human leptin system. Human leptin could work well on an agrarian diet containing large amounts of seeds from grass even though not specifically adapted to such a diet. Indeed, the ever-increasing human population today indicates that our shift to an agrarian diet is working very well, at least as measured in reproductive success. But if an agrarian diet containing large amounts of seeds from grass were a problem to the human leptin system that would mean that molecular evolution would act on the leptin system to remove such problems through adaptation. Such adaptation should be visible as genetic changes in human leptin or some other part of the leptin system. But from the molecular studies on human leptin we already know that there are no such genetic changes. However, we should not expect to find such changes in the human leptin system since the diseases of affluence usually occur late in life after reproduction and our shift to such a diet is so recent. Thus, the force of natural selection is very weak and has not had sufficient time to impose any genetic changes. But studies of molecular evolution of leptin in other animal species, which in their natural environment eat seeds from grass, might tell us if such a diet has imposed any problems to their leptin systems sufficient to cause adaptation.

Molecular evolution of leptin among rodents and birds

Further studies on the molecular evolution of leptin has shown surprisingly high similarity of leptin genes in such diverse species as mouse, rat, chicken and turkey which was ascribed to convergent or parallel evolution [43]. This high similarity was surprising since random genetic events and differing selective pressures during the long evolutionary time since the last common ancestor of bird and rodent should have turned the leptin of these species into very different varieties. Convergent molecular evolution means that natural selection has been at work on these genes and has adapted them to some similar factor(s) in the environment. Parallel evolution means that constraints on molecular evolution shared by these species due to a distant common ancestor somehow channel the molecular evolution into similar genes. Since many mammals, which also share the same distant common ancestor, do not have similar genes, it seems plausible that this high similarity is due to convergent evolution and not parallel evolution. This means that natural selection probably has adapted these genes in rodents and birds to some similar factor(s) in their environment. Diet is an important environmental factor as exemplified by primates where it affects basal metabolic rate, size, reproduction and locomotion [15]. Since leptin is a regulator of appetite, energy metabolism and reproduction it could well be subject to forces of natural selection due to diet. Except for a diet containing seeds from grass it is hard to discern an environmental characteristic shared by such diverse species as rodents and birds and sufficient to explain such high similarity of leptin genes [44-46]. Thus, a diet including large amounts of seeds from grass in rodents and birds possibly has imposed problems to their leptin systems sufficient to cause adaptation of their leptin gene. It follows that such a diet possibly imposes problems to the human leptin system, which we have concluded is not specifically adapted to such a diet. The studies on molecular evolution of leptin thus indicates that the differing results from leptin administration in humans and experimental animals could be due to adaptation of rodent leptin and insufficient adaptation of human leptin to a diet including large amounts of seeds from grass.

But what constituent(s) of seeds from grass might cause such problems to the leptin system? In the answer to this question, we believe, also lies one of the keys to understanding human leptin resistance and its related diseases. Our need now is to find some constituent which differs between seeds from grass and other plants, and which has sufficient properties to cause such problems to the human and rodent leptin system. We therefore turn to lectins.

Lectins

Lectins are “proteins possessing at least one non-catalytic domain, which binds reversibly to a specific mono- or oligosaccharide” (for most references and background see full reviews [47, 48]). That is, lectins bind specific sugar structures (i.e. carbohydrates). Lectins are abundant in the virus, bacteria, animal and plant kingdom. The ability to recognize specific sugar structures is thought to have evolved multiple times, and lectins are thus products of convergent evolution [48]. Different classes of plants contain different classes of lectins with different biochemical properties. Mono- and dicotyledonous plants have different classes of lectins, and there is a subclass of lectins only found in grasses like cereals. Many plant lectins are thought to play a role in the plants defence against being eaten. Accordingly, plant lectins have an obvious preference for binding to sugar structures of animal, fungal or microbial origin. Also consistent with this view is the finding that the highest concentrations are usually found in those plant parts which are essential for the reproductive success of the plant, such as seeds and, in particular, seed germs. One of the most intensively studied plant lectins is wheat germ agglutinin (WGA), which conveys protection against insects and fungi [48]. WGA is not only present in the seed germ, but also in gluten [49]. Gluten is the cohesive and elastic mass that remains after wheat flour dough is washed with water; gluten is a typical artefact consisting mainly of water, which can be lost upon drying, and of approximately equal amounts gliadin and glutelin trapping small percentages of albumins and globulins as well of lipids and

carbohydrates [50]. White flour consumed by humans contains a high proportion of gluten [51], which probably is better known as responsible for coeliac disease [50]. Wheat flour reportedly has agglutinating activity suggestive of lectins [52], and peptides behaving in a lectin-like manner have also been obtained upon cleavage of gliadin in gluten [50]. Sourdough lactic acid bacteria, also used in probiotics (i.e. bacteria conveying health benefits when eaten), hydrolyses these gliadin peptides and prevents their lectin-like behaviour [53]. Perhaps this property contributes to the healthy effects attributed to probiotics, whose mechanisms of action remains vastly unknown [54]. Moreover, common breakfast cereals in the US diet have been found to agglutinate red blood cells when ingested in realistic amounts, an effect most certainly attributed to WGA, other lectins and/or peptides with lectin-like behaviour [55].

Lectins are thus present in much of our food including cereal products [55, 56] and are resistant to breakdown in the gastrointestinal tract, bind to the surface epithelium of the digestive tract and can lead to anti-nutritional, mild allergic or other subclinical effects in humans and animals [47, 48]. Lectins can be transported through the gut wall into the blood circulation where they can directly influence peripheral tissues and body metabolism through binding to glycosylated structures (meaning that they have sugar structures on them), such as the insulin receptor, the epidermal growth factor receptor and the interleukin 2 receptor [56-64]. A number of effects of WGA have thus been noted such as activation of the epidermal growth factor receptor [60], mitogenesis [65], agglutination of red blood cells [47], activation of platelets and cell adhesion molecules [66], increased vascular permeability [67-69] and several effects related to autoimmunity, allergy and inflammation [56, 70]. WGA also binds to several types of mammalian cells including pancreatic duct epithelial cells [71], prostatic cancer cells [72], arterial macrophages and smooth muscle cells [73, 74] and glomerular capillary walls, mesangial cells and tubules of human kidney [58]. Furthermore, human serum contains antibodies to

the lectins of WGA, soybean and peanut [75]. Peanut lectin may contribute to the unusually strong atherogenic effect of peanut oil [76].

Lectins thus seem to have sufficient properties to possibly affect the leptin system directly, through interaction with leptin or the leptin receptor, and/or indirectly, through effects on metabolism central to the proper function of the leptin system. We will focus on the possibility of direct interactions between lectin and the leptin system.

Lectin interaction with leptin

Since lectins in the diet can enter the blood stream through the gut wall, and since many systemic proteins including membrane receptors and their binding proteins are glycosylated, it is conceivable that dietary lectins can bind to systemic proteins *in vivo* in humans. The sugar structures of a membrane receptor to which lectins could bind can be present at or close to the active ligand-binding centre of the receptor. Although the sugar structure which the lectin binds to is not the normal functional ligand binding site, the resulting conformational change and the ensuing signal transduction may be similar to that conveyed by the receptor's physiological ligand [47]. Consequently, the binding lectin can potentially mimic the effect of the physiological ligand and induce similar physiologic reactions, which has been shown for several lectins including WGA [60, 61, 77, 78]. However, the binding lectin could also block the normal functional ligand binding site, induce conformational change which blocks signal transduction, or inhibit the conformational change needed for normal function, and thus attenuate or completely abolish the normal effect of the physiological ligand [47, 64, 79-82]. Lectins could thus potentially bind to glycosylated leptin or the glycosylated leptin receptor and affect its function.

It is thought that a conformational change evolves in leptin after it is bound to the leptin receptor [83]. This conformational change may be of importance for leptin action and signalling, and could be disturbed by lectin binding. To our knowledge leptin is not glycosylated [84, 85], but

the leptin receptor is known to be [86]. Lectins binding to the leptin receptor could possibly explain the hitherto unexplained variation in reported weight of the leptin binding components from different studies which has varied from about 85 kDa, 100 and 200 kDa, 176 and 240 kDa, 280 kDa up to 450 kDa or between 200 and 670 kDa [83]. Furthermore, different leptin receptor isoforms representing different glycosylations also display different leptin binding affinity [86]. Perhaps lectins binding to sugar structures on the leptin receptor cause this variation in binding affinity in the same way as observed by Livingston and Purvis. Their study indicates that a simple interaction between WGA and the insulin receptor is sufficient to induce changes in the insulin binding properties of adipocytes [62]. Thus, it seems possible that dietary lectins could bind to at least the leptin receptor and affect its function. Should systemic leptin turn out to be glycosylated then lectins could bind to leptin and affect its function also.

The human leptin and leptin receptor gene contains a number of single nucleotide polymorphisms (SNP). Such a SNP often changes an amino acid of leptin or the extracellular region common to all isoforms of the leptin receptor. Different SNPs are associated with differences in the function of the leptin receptor, in terms of leptin binding affinity, and also with associated differences in body mass index (BMI), fat mass and leptin levels. [87-91]. There is also an association between certain SNP variants of the leptin receptor and clusters of metabolic abnormalities in response to long term overfeeding, including impaired glucose tolerance and dyslipidemia [92]. One SNP variant of leptin is associated with different fasting insulin response to exercise training [93]. Although some diseases show less or no association with SNP [89, 94-96], the above findings do strengthen the case for the clinical importance of factors affecting the function of human leptin or leptin receptor. Cereal lectins, which bind to human leptin or leptin receptor and affect their function, could thus translate into diseases of affluence.

CONCLUSIONS

The global pattern of varying prevalence of diseases of affluence suggests that some environmental factor among agrarian societies could initiate these diseases. In search of such a responsible environmental factor we have focused on the large amounts of seeds from grass, which sets an agrarian diet apart from a non-agrarian. Through previous studies in archaeology and molecular evolution we conclude that humans and human leptin are not specifically adapted to such a diet, and that leptin resistance associated with diseases of affluence could be a sign of insufficient adaptation to such a diet. We have further postulated that cereal lectins might be the cause of this leptin resistance either indirectly, through effects on metabolism central to the proper functions of the leptin system, or directly, through binding to human leptin or leptin receptor and affecting their function. The notion that dietary lectins could inhibit leptin binding implies that leptin binding affinity should be lower in leptin resistant humans on an agrarian diet. This is supported by the observations that the proportion of leptin not bound to the soluble receptor in the blood is increased in obese humans [83], and that this proportion decreases after fasting in obese but not in lean humans [97]. Further support comes from studies from our laboratory on leptin levels in populations at a transitional stage from gathering to agricultural systems [98, 99]. In addition, the recent finding that total leptin and free leptin both correlate with the dietary carbohydrate content, whereas bound leptin is associated with resting energy expenditure [100], seem to corroborate our hypothesis. This paper points to the need of further studies in this field.

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