

Agrarian diet and diseases of affluence

- Do evolutionary novel dietary lectins cause leptin resistance?

Tommy Jönsson^{1§}, Stefan Olsson², Bo Ahrén¹, Thorkild C. Bøg-Hansen³, Anita Dole³,
Staffan Lindeberg¹

¹Department of Clinical Sciences, Lund University, Lund, Sweden

²Department of Ecology, The Royal Veterinary and Agricultural University, Copenhagen, Denmark

³Institute of Molecular Pathology, University of Copenhagen, Copenhagen, Denmark

§Corresponding author

Email addresses:

TJ: tommy.jonsson@med.lu.se

SO: stefan.olsson@ecol.kvl.dk

BA: Bo.Ahren@med.lu.se

TCBH: tcbh@plab.ku.dk

AD: anita@plab.ku.dk

SL: staffan.lindeberg@med.lu.se

ABSTRACT

Background

The global pattern of varying prevalence of diseases of affluence, such as obesity, cardiovascular disease and diabetes, suggests that some environmental factor specific to agrarian societies could initiate these diseases.

Presentation of the hypothesis

We propose that a cereal-based diet could be such an environmental factor. Through previous studies in archaeology and molecular evolution we conclude that humans and the human leptin system are not specifically adapted to a cereal-based diet, and that leptin resistance associated with diseases of affluence could be a sign of insufficient adaptation to such a diet. We further propose lectins as a cereal constituent with sufficient properties to cause leptin resistance, either through effects on metabolism central to the proper functions of the leptin system, and/or directly through binding to human leptin or human leptin receptor, thereby affecting the function.

Testing the hypothesis

Prospective diet interventions should compare effects of agrarian and non-agrarian diets on incidence of diseases of affluence, their respective risk factors, and leptin resistance. A non-significant ($p=0.10$) increase of cardiovascular mortality was noted in patients advised to eat more whole-grain cereals. Our lab conducted a study on 24 domestic pigs in which a hunter-gatherer diet promoted significantly higher insulin sensitivity, lower diastolic blood pressure and lower C-reactive protein as compared to a cereal-based swine feed. Testing should also evaluate the effects of grass lectins on the leptin system *in vivo* by diet interventions, or *in vitro* in various leptin and leptin receptor models. Such studies are currently conducted by our group.

Implications of the hypothesis

If an agrarian diet initiates diseases of affluence it should be possible to identify the responsible constituents and modify or remove them so as to make agrarian diet healthier.

Background

In this paper we look at global variation in the prevalence of diseases of affluence [1], such as obesity, cardiovascular disease and diabetes type 2 [2, 3], between agrarian and non-agrarian societies. This societal division refers to differences in dietary base. The diet of an agrarian society is based on large amount of seeds from grass such as cereals (e.g. wheat, rice, maize). Cereals are per definition rare or absent in non-agrarian diet. Non-agrarian societies can be further divided into hunter-gatherer and horticultural societies. The diet of a hunter-gatherer society is based on hunting, fishing and gathering wild plants and insects. 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, which sometimes implies heavy dependence on a single starchy cultivar such as a root crop (e.g. manioc).

Global epidemiologic pattern

Among agrarian societies there is considerable variation both in time and place in the prevalence of diseases of affluence [6-8]. Among these agrarian societies some diseases of affluence, such as obesity and type 2 diabetes, are associated with increasing westernization and urbanization, although some less westernized countries such as China and countries in sub-Saharan Africa did have more cases of diabetes in rural than urban areas in 1995 [6, 7]. 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. Several risk factors for obesity and diabetes type 2, such as low

physical activity and a sedentary lifestyle with prolonged TV watching, are thus associated with westernization and urbanization, which perhaps explain their association with these diseases [9]. However, for other diseases of affluence, such as stroke and coronary heart disease (CHD), the varying prevalence among agrarian societies is puzzling with no definite association with westernization, urbanization or rise in risk factors [8, 10]. Indeed, CHD was reportedly rare in developed populations until the early 1900s with major increases in the occurrence and mortality rate from the disease in the 1930s, but with as much as five-fold differences in CHD mortality rates between European countries such as Poland and Spain [8]. A few agrarian societies, like the Amazon-dwelling Brazilian Indian tribe Amondava, reportedly lack diseases of affluence, which possibly is due to a recent and small shift in diet incorporating small amounts of cereals in an otherwise non-agrarian diet [11]. Such a pattern of differentially delayed onset of the various diseases of affluence has been described [2]. However, the global epidemiologic pattern suggests that almost all agrarian societies have some prevalence of diseases of affluence. In contrast, diseases of affluence have been virtually absent among many non-agrarian societies in Melanesia, Malaysia, Africa, South America and the Arctic [2, 5, 12]. One such traditional population are the horticultural Trobriand Islanders with a mortality from atherothrombotic circulatory diseases 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 [2, 12]. These disproportionate differences in prevalence between agrarian and non-agrarian societies differ even more when considering only the incidence of non-infectious stroke [10, 13]. Moreover, when people living in non-agrarian societies migrate to an agrarian society or when their own society becomes agrarian they contract diseases of affluence [2, 12], which illustrate the general rule that there is no genetic protection against diseases of affluence, only genetic variation in degree of susceptibility [2].

The global epidemiologic pattern of varying prevalence of diseases of affluence thus suggests that some environmental factor specific to agrarian societies could initiate these diseases. Obviously, there are many such candidate environmental factors, but in this paper we study the cereals, the clearest defining dietary difference between an agrarian and non-agrarian diet. Since nothing in biology makes sense except in the light of evolution [14], we look at the cereal component of human diet from an evolutionary perspective.

Human diet and evolution

The grasses emerged between 65 and 55 million years ago (mya) [15]. Since the last common ancestor of living primates, including humans, emerged before this time, some 90 to 65 mya, it cannot have had a diet consisting of seeds from grass [16, 17]. Subsequent evolution of our primate ancestors up until 4-8 mya is thought to have taken place in the trees [18-21], where almost all potential plant food comes from dicotyledonous species [22] and the monocotyledonous grasses are absent [15]. The archaeological evidence during the last four million years of evolution towards *Homo sapiens* suggests that if grass seeds were being incorporated into the diet of our ancestors, they probably only contributed a small part [23]. *Homo sapiens* emerged about 200,000 years ago [24, 25], and seeds from grass were probably not key dietary staples of *Homo sapiens* hunter-gatherers [26]. About 10,000 years ago (e.g. 500 generations) some populations invented agriculture [26], and these possibly have some genetic adaptation to an agrarian diet such as lower prevalence of celiac disease and related HLA genotypes [27, 28]. However, many populations shifted to agrarian diet more recently, between 1-100 generations ago, which from an evolutionary perspective is too short time to admit adaptation [29]. Thus, when examining human diet from an evolutionary perspective, it makes sense that humans with an evolutionary novel agrarian diet could suffer from diseases of affluence due to insufficient adaptation [29]. Obviously, many metabolic factors and

pathways are important in the onset and development of diseases of affluence. When looking for metabolic signs of such insufficient adaptation, one of the more relevant associations is that between diseases of affluence and leptin resistance, an acquired insensitivity to high levels of leptin [30-36].

Leptin resistance

Leptin acts as a signal to the brain to inhibit food intake and enable the storage in adipocytes of surplus calories while simultaneously protecting peripheral non-adipose tissue from toxic effects of intracellular lipid overload [37]. Leptin also effects the growth of blood vessel and bone; the immune system; glucose- and fat metabolism and the reproductive system [30, 38]. Leptin administered peripherally in animal models such as rodents promotes weight loss and satiation, but peripheral administration of leptin in obese human does not promote significant weight loss [30, 39]. This difference in effect together with the observation that most obese humans have high levels of leptin suggest that leptin resistance causes human obesity [30, 39]. Sometimes end-organ resistance can be caused by mutations in hormone receptors, which has been described for several hormones. The pathophysiology of acquired forms of end-organ resistance such as insulin and leptin resistance has been elusive [40]. 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 [41]. This difference could be genetically based and possibly an adaptation of the human or experimental animal leptin system to some environmental factors affecting their respective ancient ancestors. But this difference could also be due to an insufficient adaptation to some environmental factors, which are 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.

Molecular evolution of leptin

The hominoids (gibbon, orang-utan, gorilla, chimpanzee, early human and modern human) emerged 25-30 mya [17]. Studies on leptin molecular evolution have shown a significant increase of non-synonymous to synonymous changes [29] 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 [41]. This implies that the ancestral line of primates giving rise to hominoids probably acquired several positive non-synonymous changes of their leptin gene due to adaptations, and that the leptin genes of humans have not changed much since the emergence of hominoids. Thus, based on findings from previous section on human diet and evolution, it is very unlikely that human leptin could be specifically adapted to an agrarian diet. Furthermore, similar studies on molecular evolution have shown high similarity of leptin genes in such diverse species as mouse, rat, chicken and turkey, which was ascribed to convergent or parallel evolution [42]. Since many mammals, which share the same distant common ancestor with these species, do not have similar genes, it seems plausible that this high similarity is due to convergent evolution and not parallel evolution [29]. This implies that natural selection has caused the leptin genes of these bird and rodent species to be highly similar by adapting them to some similar factor(s) in the environment of their ancient ancestors. Diet is an important environmental factor, as exemplified by primates, where it affects basal metabolic rate, size, reproduction and locomotion [17]. 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 diverse rodent and bird species and sufficient to explain such high similarity of leptin genes [43-45]. Thus, it is possible that leptin of these

rodent and bird species are specifically adapted to a diet including large amounts of seeds from grass. 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 indicate that the differing results from leptin administration in humans and experimental animals could be due to adaptation of mouse and rat leptin and insufficient adaptation of human leptin to a diet including large amounts of seeds from grass. When looking for constituents of seeds from grass explaining these differences, we find the properties of lectins interesting.

Lectins

Lectins are proteins abundant in the virus, bacteria, animal and plant kingdom, which binds reversibly to specific sugar structures (for most references and background see [46, 47]). Different classes of plants, such as mono- and dicotyledonous, have different classes of lectins with differing biochemical properties, 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, and are usually at highest concentrations in plant parts essential for reproductive success such as seed germs. The intensively studied lectin wheat germ agglutinin (WGA), which protects against insects and fungi [47], is present in wheat seed in both the germ and the gluten part of endosperm [48]. Peptides behaving in a lectin-like manner have also been obtained upon cleavage of gliadin in gluten [49]. Sourdough lactic acid bacteria hydrolyse gliadin peptides and inhibit their lectin-like behaviour [50], which perhaps explains the unexplained healthy effects of probiotics [51]. White flour consumed by humans contains a high proportion of gluten and has agglutinating activity suggestive of lectins [52-55]. Thus, lectins are present in our food, they are resistant to breakdown in the

gastrointestinal tract, they bind to the surface epithelium of the digestive tract and they can lead to anti-nutritional, mild allergic or other subclinical effects in humans and animals [46, 47]. Lectins can also be transported through the gut wall into the blood circulation, where they directly influence peripheral tissues and body metabolism through the binding to glycosylated structures, such as the insulin receptor, the epidermal growth factor receptor and the interleukin 2 receptor [55-63]. WGA have effects on activation of the epidermal growth factor receptor [59], mitogenesis [64], agglutination of red blood cells [46], activation of platelets and cell adhesion molecules [65], increased vascular permeability [66-68] and several effects related to autoimmunity, allergy and inflammation [55, 69]. WGA also binds to several types of mammalian cells including pancreatic duct epithelial cells [70], prostatic cancer cells [71], arterial macrophages and smooth muscle cells [72, 73], glomerular capillary walls, mesangial cells and tubules of human kidney [57]. Human serum contains antibodies against WGA and lectins of soybean and peanut [74]. Hence, lectins have sufficient properties to affect the leptin system indirectly, through effects on metabolism central to the proper function of the leptin system, and/or directly, through interaction with leptin or the leptin receptor. The intriguing possibility of direct interaction between lectin and the leptin system is worth some additional comments.

Possible direct interaction between lectin and the leptin system

Lectins binding to sugar structures of a membrane receptor can mimic or block the effect of the physiological ligand [46, 59, 60, 63, 75-80]. Leptin is not glycosylated, but the leptin receptor is and lectins binding to different leptin receptor glycosylations might explain different leptin binding affinity [81-83], as observed by Livingston and Purvis in their study on WGA and the insulin receptor [61]. Thus, dietary lectins could possibly bind to the leptin receptor and affect its function, which could translate into diseases of affluence as indicated

by studies on effects of single nucleotide polymorphisms on function of leptin and the leptin receptor [84-90].

Presentation of the hypothesis

The global pattern of varying prevalence of diseases of affluence suggests that some environmental factor specific to agrarian societies could initiate these diseases [2, 12]. We propose that cereals, the clearest defining dietary difference between an agrarian and non-agrarian diet, could be such an environmental factor. Through previous studies in archaeology [15-23, 26, 29] and molecular evolution [41, 42] we conclude that humans and human leptin system are not specifically adapted to a cereal-based diet, and that leptin resistance associated with diseases of affluence [30-36] could indicate insufficient adaptation to such a diet. As for the constituent(s) of cereals causing leptin resistance as a sign of insufficient adaptation, we propose lectins as a candidate with sufficient properties. Cereal lectins are specific to cereals [46, 47], they are present in our food [48, 49, 52-55], they enter our systemic circulation and have many reported effects in our body including the binding to receptors, such as the insulin receptor, the epidermal growth factor receptor and the interleukin 2 receptor [46, 55-73]. Cereal lectins could thus cause leptin resistance either indirectly, through effects on metabolism central to the proper functions of the leptin system, and/or directly, through binding to human leptin or leptin receptor, thereby affecting the function. The intriguing possibility of direct interaction between lectin and the leptin receptor could alter the function of the leptin receptor and translate into diseases of affluence [46, 59-61, 63, 75-80, 83-90].

Testing the hypothesis

The hypothesis that an agrarian diet could initiate diseases of affluence should ideally be tested in prospective diet interventions comparing this diet with non-agrarian diets. Hard end-

points should be various diseases of affluence and soft end-points their respective risk factors, specifically including leptin resistance. The only relevant human prospective intervention trial that we are aware of found a non-significant ($p=0.10$) increase of cardiovascular mortality in CHD patients who were advised to eat more whole-grain cereals compared to those who were not advised to eat more whole-grain cereals [91]. We performed a trial on 24 domestic pigs in which a cereal-free hunter-gatherer diet promoted significantly higher insulin sensitivity, lower diastolic blood pressure and lower C-reactive protein as compared to a cereal-based swine feed (in manuscript). A prospective observational study on intake of refined grains as part of a “western diet” pattern showed a positive association with increased risk for type 2 diabetes [92]. Although the foods with major contributions to the “western diet” pattern were all positively associated with increased risk for type 2 diabetes, the consumption of refined grains remained significantly associated with the risk for type 2 diabetes when the foods with major contributions were modelled simultaneously [92]. However, the same study also showed a reduced risk for type 2 diabetes with a high intake of whole grain as part of a “prudent” diet pattern [92], and whole grains are reportedly also inversely related to weight gain, even after multivariate analysis for several indicators of a healthy living such as non-smoking and physical activity [93]. Accordingly, there are contradictory results from studies on effects of cereal grains on diseases of affluence. This is possibly due to beneficial effects from whole grain as compared to refined grain, such as higher fibre and micronutrient content, coupled with the usually inverse relationship between intake of whole and refined grain [93].

Evaluating the effects of grass lectins on the leptin system *in vivo* by diet interventions or *in vitro* in various leptin and/or leptin receptor models could test the hypothesis that cereal lectins might be the cause of leptin resistance. Such studies are currently conducted by our group.

Implications of the hypothesis

If an agrarian diet initiates diseases of affluence it should be possible to identify the responsible constituents and modify or remove them so as to make agrarian diet healthier. Furthermore, in animal experiments, the possible species-specific differences in adaptation to diets outlined in this article and their effects on studied parameters should be kept in mind when choosing the animal and the animal feed for the study. Furthermore, if cereal lectins should appear to have significant effects on human metabolism, then it is suggested that other plant lectins like peanut-lectin should be investigated in this regard as well. If dietary lectins could inhibit leptin binding, then 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 [94], and that this proportion decreases after fasting in obese but not in lean humans [95]. Further support comes from studies from our laboratory on leptin levels in populations at a transitional stage from gathering to agricultural systems [96, 97]. 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 [98], seem to support our hypothesis.

Competing interests

None

Authors' contributions

TJ conceived of and wrote the article. SO and SL conceived of and participated in the design of the article, and revised it critically for important intellectual content. BA, TB and AD have

been involved in drafting the manuscript and revising it critically for important intellectual content. All authors read and approved the final manuscript.

Acknowledgements

None

References

1. McKeown T: *The origins of human disease*. Basil Blackwell; 1988.
2. Trowell HC, Burkitt DP (Eds.): **Western diseases: their emergence and prevention**. London: Edward Arnold; 1981.
3. Temple NJ, Burkitt DP: *Western diseases : their dietary prevention and reversibility*. Totowa, N.J.: Humana Press; 1994.
4. Semaw S, Rogers MJ, Quade J, Renne PR, Butler RF, Dominguez-Rodrigo M, Stout D, Hart WS, Pickering T, Simpson SW: **2.6-Million-year-old stone tools and associated bones from OGS-6 and OGS-7, Gona, Afar, Ethiopia**. *J Hum Evol* 2003, **45**:169-177.
5. Lindeberg S, Cordain L, Eaton B: **Biological and Clinical Potential of a Palaeolithic Diet**. *Journal of Nutritional & Environmental Medicine* 2003, **13**:149-160.
6. King H, Aubert RE, Herman WH: **Global burden of diabetes, 1995-2025: prevalence, numerical estimates, and projections**. *Diabetes Care* 1998, **21**:1414-1431.
7. van Dam RM: **The epidemiology of lifestyle and risk for type 2 diabetes**. *Eur J Epidemiol* 2003, **18**:1115-1125.
8. Walker AR, Walker BF, Segal I: **Some puzzling situations in the onset, occurrence and future of coronary heart disease in developed and developing populations, particularly such in sub-Saharan Africa**. *J R Soc Health* 2004, **124**:40-46.
9. Hu FB, Leitzmann MF, Stampfer MJ, Colditz GA, Willett WC, Rimm EB: **Physical activity and television watching in relation to risk for type 2 diabetes mellitus in men**. *Arch Intern Med* 2001, **161**:1542-1548.
10. Feigin VL, Lawes CM, Bennett DA, Anderson CS: **Stroke epidemiology: a review of population-based studies of incidence, prevalence, and case-fatality in the late 20th century**. *Lancet Neurol* 2003, **2**:43-53.
11. Pavan L, Casiglia E, Braga LM, Winnicki M, Puato M, Pualetto P, Pessina AC: **Effects of a traditional lifestyle on the cardiovascular risk profile: the Amondava population of the Brazilian Amazon. Comparison with matched African, Italian and Polish populations**. *J Hypertens* 1999, **17**:749-756.
12. Lindeberg S: **Apparent absence of cerebrocardiovascular disease in Melanesians. Risk factors and nutritional considerations - the Kitava Study**. *PhD Thesis*. University of Lund, 1994.
13. Lindeberg S: **Stroke in Papua New Guinea**. *Lancet Neurol* 2003, **2**:273; discussion 273.

14. Dobzhansky T: **Nothing in Biology Makes Sense Except in the Light of Evolution.** *American Biology Teacher* 1973, **35**:125-129.
15. Willis KJ, McElwain JC: *The evolution of plants*. Oxford: Oxford University Press; 2002.
16. Sargis EJ: **Paleontology. Primate origins nailed.** *Science* 2002, **298**:1564-1565.
17. Jones S, Martin R, Pilbeam D (Eds.): **The Cambridge encyclopedia of human evolution.** Cambridge: Cambridge University Press; 1992.
18. Wood B, Brooks A: **Human evolution. We are what we ate.** *Nature* 1999, **400**:219-220.
19. Preuschoft H: **Mechanisms for the acquisition of habitual bipedality: are there biomechanical reasons for the acquisition of upright bipedal posture?** *J Anat* 2004, **204**:363-384.
20. Pickford M: **Palaeoenvironments and hominoid evolution.** *Z Morphol Anthropol* 2002, **83**:337-348.
21. Bramble DM, Lieberman DE: **Endurance running and the evolution of Homo.** *Nature* 2004, **432**:345-352.
22. Milton K: **Primate Diets and Gut Morphology: Implications for Hominid Evolution.** In *Food and evolution : toward a theory of human food habits : 94th; Philadelphia*. Edited by Ross Eric B, Harris M, Wenner-Gren Foundation for Anthropological R. Temple University Press; 1987: pp 93-115.
23. Richards MP: **A brief review of the archaeological evidence for Palaeolithic and Neolithic subsistence.** *Eur J Clin Nutr* 2002, **56**:16 p following 1262.
24. McDougall I, Brown FH, Fleagle JG: **Stratigraphic placement and age of modern humans from Kibish, Ethiopia.** *Nature* 2005, **433**:733-736.
25. Ingman M, Kaessmann H, Paabo S, Gyllensten U: **Mitochondrial genome variation and the origin of modern humans.** *Nature* 2000, **408**:708-713.
26. Kuhn SL, Stiner MC: **The Antiquity of Hunter-gatherers.** In *Hunter-gatherers : an interdisciplinary perspective*. Edited by Panter-Brick C, Rowley-Conwy P, Layton R. Cambridge: Cambridge University Press; 2001: pp 99-142: *The Biosocial Society symposium series*].
27. Cronin CC, Shanahan F: **Why is celiac disease so common in Ireland?** *Perspect Biol Med* 2001, **44**:342-352.
28. Rostami K, Malekzadeh R, Shahbazkhani B, Akbari MR, Catassi C: **Coeliac disease in Middle Eastern countries: a challenge for the evolutionary history of this complex disorder?** *Dig Liver Dis* 2004, **36**:694-697.
29. Freeman S, Herron JC: *Evolutionary analysis*. 3rd ed. edn: Upper Saddle River; 2004.
30. Veniant MM, LeBel CP: **Leptin: from animals to humans.** *Curr Pharm Des* 2003, **9**:811-818.
31. El-Haschimi K, Lehnert H: **Leptin resistance - or why leptin fails to work in obesity.** *Exp Clin Endocrinol Diabetes* 2003, **111**:2-7.
32. El-Haschimi K, Pierroz DD, Hileman SM, Bjorbaek C, Flier JS: **Two defects contribute to hypothalamic leptin resistance in mice with diet-induced obesity.** *J Clin Invest* 2000, **105**:1827-1832.
33. Halaas JL, Boozer C, Blair-West J, Fidahusein N, Denton DA, Friedman JM: **Physiological response to long-term peripheral and central leptin infusion in lean and obese mice.** *Proc Natl Acad Sci U S A* 1997, **94**:8878-8883.
34. Jequier E: **Leptin signaling, adiposity, and energy balance.** *Ann N Y Acad Sci* 2002, **967**:379-388.

35. Frederich RC, Hamann A, Anderson S, Lollmann B, Lowell BB, Flier JS: **Leptin levels reflect body lipid content in mice: evidence for diet-induced resistance to leptin action.** *Nat Med* 1995, **1**:1311-1314.
36. Maffei M, Halaas J, Ravussin E, Pratley RE, Lee GH, Zhang Y, Fei H, Kim S, Lallone R, Ranganathan S, et al.: **Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects.** *Nat Med* 1995, **1**:1155-1161.
37. Unger RH: **Longevity, lipotoxicity and leptin: the adipocyte defense against feasting and famine.** *Biochimie* 2005, **87**:57-64.
38. Trayhurn P: **Leptin--a critical body weight signal and a "master" hormone?** *Sci STKE* 2003, **2003**:PE7.
39. Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, Ohannesian JP, Marco CC, McKee LJ, Bauer TL: **Serum immunoreactive-leptin concentrations in normal-weight and obese humans [see comments].** *N Engl J Med* 1996, **334**:292-295.
40. Jameson JL: **Molecular mechanisms of end-organ resistance.** *Growth Horm IGF Res* 2004, **14 Suppl A**:S45-50.
41. Gaucher EA, Miyamoto MM, Benner SA: **Evolutionary, structural and biochemical evidence for a new interaction site of the leptin obesity protein.** *Genetics* 2003, **163**:1549-1553.
42. Doyon C, Drouin G, Trudeau VL, Moon TW: **Molecular evolution of leptin.** *Gen Comp Endocrinol* 2001, **124**:188-198.
43. Hoyo Josep d, Arlott N, Elliott A, Sargatal J, Bierregaard R: *Handbook of the birds of the world.* Barcelona: Lynx Edicions; 1994.
44. Nowak Ronald M, Walker Ernest P: *Walker's mammals of the world.* 5th ed edn. Baltimore: Johns Hopkins University Press; 1991.
45. Corbet GB, Harris S, Mammal S: *The handbook of British mammals.* 3rd. ed. edn. Oxford: Blackwell Scientific; 1991.
46. Van Damme JME: *Handbook of plant lectins : properties and biomedical applications.* Chichester: John Wiley; 1998.
47. Sharon N, Lis H: *Lectins.* 2nd ed. edn. Dordrecht ; London: Kluwer Academic Publishers; 2003.
48. Kolberg J, Wedege E, Sollid L: **Immunoblotting detection of lectins in gluten and white rice flour.** *Biochem Biophys Res Commun* 1987, **142**:717-723.
49. Silano M, De Vincenzi M: **Bioactive antinutritional peptides derived from cereal prolamins: a review.** *Nahrung* 1999, **43**:175-184.
50. Di Cagno R, De Angelis M, Lavermicocca P, De Vincenzi M, Giovannini C, Faccia M, Gobbetti M: **Proteolysis by sourdough lactic acid bacteria: effects on wheat flour protein fractions and gliadin peptides involved in human cereal intolerance.** *Appl Environ Microbiol* 2002, **68**:623-633.
51. Mercenier A, Pavan S, Pot B: **Probiotics as biotherapeutic agents: present knowledge and future prospects.** *Curr Pharm Des* 2003, **9**:175-191.
52. Shewry PR, Halford NG: **Cereal seed storage proteins: structures, properties and role in grain utilization.** *J Exp Bot* 2002, **53**:947-958.
53. Minetti M, Aducci P, Teichner A: **A new agglutinating activity from wheat flour inhibited by tryptophan.** *Biochim Biophys Acta* 1976, **437**:505-517.
54. Nachbar MS, Oppenheim JD: **Lectins in the United States diet: a survey of lectins in commonly consumed foods and a review of the literature.** *Am J Clin Nutr* 1980, **33**:2338-2345.

55. Freed DLJ: **Lectins in food: Their importance in health and disease.** *Journal of Nutritional Medicine* 1991, **2**:45-65.
56. Wang Q, Yu LG, Campbell BJ, Milton JD, Rhodes JM: **Identification of intact peanut lectin in peripheral venous blood.** *Lancet* 1998, **352**:1831-1832.
57. Freed DL: **Do dietary lectins cause disease?** *Bmj* 1999, **318**:1023-1024.
58. Wang XY, Bergdahl K, Heijbel A, Liljebris C, Bleasdale JE: **Analysis of in vitro interactions of protein tyrosine phosphatase 1B with insulin receptors.** *Mol Cell Endocrinol* 2001, **173**:109-120.
59. Zeng FY, Benguria A, Kafert S, Andre S, Gabius HJ, Villalobo A: **Differential response of the epidermal growth factor receptor tyrosine kinase activity to several plant and mammalian lectins.** *Mol Cell Biochem* 1995, **142**:117-124.
60. Ponzio G, Debant A, Contreres JO, Rossi B: **Wheat-germ agglutinin mimics metabolic effects of insulin without increasing receptor autophosphorylation.** *Cell Signal* 1990, **2**:377-386.
61. Livingston JN, Purvis BJ: **The effects of wheat germ agglutinin on the adipocyte insulin receptor.** *Biochim Biophys Acta* 1981, **678**:194-201.
62. Hedo JA, Harrison LC, Roth J: **Binding of insulin receptors to lectins: evidence for common carbohydrate determinants on several membrane receptors.** *Biochemistry* 1981, **20**:3385-3393.
63. Fujii M, Sugamura K, Nakamura M, Ishii T, Hinuma Y: **Selective inhibition of high- but not low-affinity interleukin 2 binding by lectins and anti-interleukin 2 receptor alpha antibody.** *Microbiol Immunol* 1988, **32**:857-867.
64. Kilpatrick DC: **Mechanisms and assessment of lectin-mediated mitogenesis.** *Mol Biotechnol* 1999, **11**:55-65.
65. Ohmori T, Yatomi Y, Wu Y, Osada M, Satoh K, Ozaki Y: **Wheat germ agglutinin-induced platelet activation via platelet endothelial cell adhesion molecule-1: involvement of rapid phospholipase C gamma 2 activation by Src family kinases.** *Biochemistry* 2001, **40**:12992-13001.
66. Banks WA, Ibrahim F, Farr SA, Flood JF, Morley JE: **Effects of wheatgerm agglutinin and aging on the regional brain uptake of HIV-1GP120.** *Life Sci* 1999, **65**:81-89.
67. Villegas JC, Broadwell RD: **Transcytosis of protein through the mammalian cerebral epithelium and endothelium. II. Adsorptive transcytosis of WGA-HRP and the blood-brain and brain-blood barriers.** *J Neurocytol* 1993, **22**:67-80.
68. Mares V, Borges LF, Sidman RL: **Uptake and transport of lectins from the cerebrospinal fluid by cells of the immature mouse brain.** *Acta Histochem* 1984, **74**:11-19.
69. Cordain L, Toohey L, Smith MJ, Hickey MS: **Modulation of immune function by dietary lectins in rheumatoid arthritis.** *Br J Nutr* 2000, **83**:207-217.
70. Geleff S, Bock P: **Pancreatic duct glands. II. Lectin binding affinities of ductular epithelium, ductular glands, and Brunner glands.** *Histochemistry* 1984, **80**:31-38.
71. Gabor F, Klausegger U, Wirth M: **The interaction between wheat germ agglutinin and other plant lectins with prostate cancer cells Du-145.** *Int J Pharm* 2001, **221**:35-47.
72. Davis HR, Glagov S: **Lectin binding to distinguish cell types in fixed atherosclerotic arteries.** *Atherosclerosis* 1986, **61**:193-203.
73. Kagami H, Uryu K, Okamoto K, Sakai H, Kaneda T, Sakanaka M: **Differential lectin binding on walls of thoraco-cervical blood vessels and lymphatics in rats.** *Okajimas Folia Anat Jpn* 1991, **68**:161-170.

74. Tchernychev B, Wilchek M: **Natural human antibodies to dietary lectins.** *FEBS Lett* 1996, **397**:139-142.
75. Shechter Y: **Bound lectins that mimic insulin produce persistent insulin-like activities.** *Endocrinology* 1983, **113**:1921-1926.
76. Cuatrecasas P, Tell GP: **Insulin-like activity of concanavalin A and wheat germ agglutinin--direct interactions with insulin receptors.** *Proc Natl Acad Sci U S A* 1973, **70**:485-489.
77. Damm I, Mikkat U, Kirchhoff F, Bockmann S, Jonas L: **Inhibitory effect of the lectin wheat germ agglutinin on the binding of 125I-CCK-8s to the CCK-A and -B receptors of AR42J cells.** *Pancreas* 2004, **28**:31-37.
78. Lascols O, Cherqui G, Munier A, Picard J, Capeau J: **Differences in both glycosylation and binding properties between rat and mouse liver prolactin receptors.** *Cell Mol Biol (Noisy-le-grand)* 1994, **40**:359-371.
79. Chochola J, Fabre C, Bellan C, Luis J, Bourgerie S, Abadie B, Champion S, Marvaldi J, el Battari A: **Structural and functional analysis of the human vasoactive intestinal peptide receptor glycosylation. Alteration of receptor function by wheat germ agglutinin.** *J Biol Chem* 1993, **268**:2312-2318.
80. Johnson RJ, Simpson S, Van Epps DE, Chenoweth DE: **Wheat germ agglutinin inhibits the C5a receptor interaction: implications for receptor microheterogeneity and ligand binding site.** *J Leukoc Biol* 1992, **52**:3-10.
81. Elliott S, Lorenzini T, Asher S, Aoki K, Brankow D, Buck L, Busse L, Chang D, Fuller J, Grant J, et al: **Enhancement of therapeutic protein in vivo activities through glycoengineering.** *Nat Biotechnol* 2003, **21**:414-421.
82. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM: **Positional cloning of the mouse obese gene and its human homologue.** *Nature* 1994, **372**:425-432.
83. Lammert A, Brockmann G, Renne U, Kiess W, Bottner A, Thiery J, Kratzsch J: **Different isoforms of the soluble leptin receptor in non-pregnant and pregnant mice.** *Biochem Biophys Res Commun* 2002, **298**:798-804.
84. Quinton ND, Lee AJ, Ross RJ, Eastell R, Blakemore AI: **A single nucleotide polymorphism (SNP) in the leptin receptor is associated with BMI, fat mass and leptin levels in postmenopausal Caucasian women.** *Hum Genet* 2001, **108**:233-236.
85. Thompson DB, Ravussin E, Bennett PH, Bogardus C: **Structure and sequence variation at the human leptin receptor gene in lean and obese Pima Indians.** *Hum Mol Genet* 1997, **6**:675-679.
86. Stefan N, Vozarova B, Del Parigi A, Ossowski V, Thompson DB, Hanson RL, Ravussin E, Tataranni PA: **The Gln223Arg polymorphism of the leptin receptor in Pima Indians: influence on energy expenditure, physical activity and lipid metabolism.** *Int J Obes Relat Metab Disord* 2002, **26**:1629-1632.
87. Chagnon YC, Chung WK, Perusse L, Chagnon M, Leibel RL, Bouchard C: **Linkages and associations between the leptin receptor (LEPR) gene and human body composition in the Quebec Family Study.** *Int J Obes Relat Metab Disord* 1999, **23**:278-286.
88. Wauters M, Mertens I, Chagnon M, Rankinen T, Considine RV, Chagnon YC, Van Gaal LF, Bouchard C: **Polymorphisms in the leptin receptor gene, body composition and fat distribution in overweight and obese women.** *Int J Obes Relat Metab Disord* 2001, **25**:714-720.
89. Ukkola O, Tremblay A, Despres JP, Chagnon YC, Campfield LA, Bouchard C: **Leptin receptor Gln223Arg variant is associated with a cluster of metabolic**

- abnormalities in response to long-term overfeeding.** *J Intern Med* 2000, **248**:435-439.
90. Lakka TA, Rankinen T, Weisnagel SJ, Chagnon YC, Lakka HM, Ukkola O, Boule N, Rice T, Leon AS, Skinner JS, et al: **Leptin and leptin receptor gene polymorphisms and changes in glucose homeostasis in response to regular exercise in nondiabetic individuals: the HERITAGE family study.** *Diabetes* 2004, **53**:1603-1608.
 91. Burr ML, Fehily AM, Gilbert JF, Rogers S, Holliday RM, Sweetnam PM, Elwood PC, Deadman NM: **Effects of changes in fat, fish, and fibre intakes on death and myocardial reinfarction: diet and reinfarction trial (DART) [see comments].** *Lancet* 1989, **2**:757-761.
 92. van Dam RM, Rimm EB, Willett WC, Stampfer MJ, Hu FB: **Dietary patterns and risk for type 2 diabetes mellitus in U.S. men.** *Ann Intern Med* 2002, **136**:201-209.
 93. Koh-Banerjee P, Franz M, Sampson L, Liu S, Jacobs DR, Jr., Spiegelman D, Willett W, Rimm E: **Changes in whole-grain, bran, and cereal fiber consumption in relation to 8-y weight gain among men.** *Am J Clin Nutr* 2004, **80**:1237-1245.
 94. Wu Z, Bidlingmaier M, Liu C, De Souza EB, Tschop M, Morrison KM, Strasburger CJ: **Quantification of the soluble leptin receptor in human blood by ligand-mediated immunofunctional assay.** *J Clin Endocrinol Metab* 2002, **87**:2931-2939.
 95. Landt M, Horowitz JF, Coppack SW, Klein S: **Effect of short-term fasting on free and bound leptin concentrations in lean and obese women.** *J Clin Endocrinol Metab* 2001, **86**:3768-3771.
 96. Lindgarde F, Ercilla MB, Correa LR, Ahren B: **Body adiposity, insulin, and leptin in subgroups of peruvian amerindians.** *High Alt Med Biol* 2004, **5**:27-31.
 97. Lindgarde F, Widen I, Gebb M, Ahren B: **Traditional versus agricultural lifestyle among Shuar women of the Ecuadorian Amazon: effects on leptin levels.** *Metabolism* 2004, **53**:1355-1358.
 98. Magni P, Liuzzi A, Ruscica M, Dozio E, Ferrario S, Bussi I, Minocci A, Castagna A, Motta M, Savia G: **Free and bound plasma leptin in normal weight and obese men and women: relationship with body composition, resting energy expenditure, insulin-sensitivity, lipid profile and macronutrient preference.** *Clin Endocrinol (Oxf)* 2005, **62**:189-196.