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Leading article

Leptin, the adipocyte hormone

Acting in concert with many partners leptin regulates body weight, reproduction, bone remodelling and many other physiological processes

Existence of a humoral factor of peripheral tissue origin playing a pivotal role in regulating body weight was suggested nearly 50 years ago, but attempts at identifying it using conventional biochemical tools were unsuccessful. After the advent of recombinant DNA technology, Jeffrey Friedman's group cloned the gene responsible for this putative humoral factor in 1994 (1). The gene was named ob (ob in rodents and the human homologue OB) as it was cloned initially from the genetically obese ob/ob mouse. The protein product was named leptin ("leptos" is the Greek word for thin). Leptin was greeted with much enthusiasm as a potential anti-obesity wonder drug, but when leptin resistance rather than leptin deficiency was noted in most instances of human obesity (2) these hopes receded. Proliferation of research on leptin that followed in the next few years established leptin not only as a hormone regulating body weight and energy expenditure, but also as a molecular conductor in an orchestra signalling nutritional status of an organism to a number of physiological processes (3). This paper addresses only a few selected aspects of leptin.

Leptin is a helical cytokine belonging to the tumour necrosis factor group. It is synthesised as a 167 amino acid protein, and after cleavage of the secretory signal, it circulates in blood as a 146 amino acid protein either in the free form or bound to proteins. Plasma leptin level reflects adiposity (2) but there is a gender variation even after adiposity is accounted for (4). An inhibitory effect of androgens rather than a stimulatory effect of oestrogen is the likely cause of this gender variation (5). Leptin shows a diurnal variation, with peak levels about 02.00 h, and pulsatile secretion (6,7).The nocturnal rise in leptin resembles that of prolactin. Acute changes in metabolic status also affect leptin secretion. Fasting decreases and re-feeding restores serum levels (8).

Leptin is synthesised in adipose tissue, white adipose tissue being the predominant site. Subcutaneous adipocytes synthesise more leptin than omental adipocytes. Placenta is a major site of synthesis during pregnancy (9). Leptin synthesis at low levels occurs in several other tissues. Insulin, glucocorticoids, increased size of adipocytes and some cytokines (10,11,12) are known stimulators of leptin synthesis. The exponential increase in leptin secretion with increasing adiposity is attributed to increased leptin synthesis by larger adipocytes (2). Anorexia seen in systemic diseases is at least partly mediated by increased leptin secretion in response to cytokines (eg. in cancer) or by reduced renal clearance (eg. in chronic renal disease) (5). Inhibitors of leptin synthesis include fasting, androgens, sympathetic activity acting *via* 03 adrenoceptors, cyclic AMP and thiazolidinediones.

Stephen Lock MD, FRCP
London, UK.

Samiran Nundy FRCS, FRCP
New Delhi, India.

N Medappa MD
New Delhi, India.

Jane Smith BA, MSc
London, UK.

S K Sarin MD, DM
New Delhi, India.

David Warrell MD, FRCP
Oxford, UK.

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Wijerama House
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E-mail anpress@sltnet.lk

Leptin receptors were first identified in the mouse choroid plexus (13). Leptin receptor is transcribed by OB-R or DB gene. Alternative splicing yields several isoforms distributed in the hypothalamus, other areas of the brain and in a number of peripheral tissues. Of these the OB-Rb isoform has the full length of the cytoplasmic domain, is abundant in the hypothalamus and mediates signal transduction. Other leptin receptor isoforms have variously truncated or absent cytoplasmic domains. Some of these facilitate transport of leptin - for example, across the blood brain barrier. Isoforms lacking the cytoplasmic domain probably function as soluble receptors. OB-Rb form uses Jak-Stat pathway for signal transduction, a pathway used by other cytokines. Current evidence suggests that the OB-Ra isoform despite having a shorter cytoplasmic domain mediates signal transduction using alternative pathways.

Several genetically obese rodent models have mutations either in the leptin gene or in the leptin receptor gene. These animals were extensively used intially to clone the genes for leptin and its receptor, and later to understand physiological function of leptin (1, 13, 14). The ob/ob mouse has a mutated leptin gene, one strain fails to transcribe DNA, and the other strain synthesises a truncated protein with no biological effect. They are massively obese, hyperphagic and sterile. Exogenous leptin reduces their appetite, increases energy expenditure and corrects sterility (14,15). The db/db mouse has a mutated leptin receptor and is insensitive to leptin (14). Though rare, mutations in the leptin gene (16) and in the leptin receptor gene (17) may lead to morbid obesity in humans.

It has been postulated that the "set point" of body weight is regulated by a lower leptin level in the lean and a higher leptin level in the obese. Thus, weight loss following an illness will reduce leptin levels, and the resulting increased food intake and reduced energy expenditure will restore the adipocyte mass and leptin levels to the pre-determined levels. The same mechanism poses problems for obese persons who want to lose weight (10). Though human obesity is often associated with leptin resistance about 5 to 10% of obese individuals have relatively low levels of leptin. A web posting from Howard Hughes Medical Institute reports, "early indications from clinical trials underway are that some obese humans may lose weight on leptin therapy". Leptin receptor immunoreactivity has been co-localised in a number of hypothalamic nuclei secreting neurotransmitters and neuropeptides such as neuropeptide Y (NPY), proopiomelanocortin (POMC) and melanin concentrating hormone (MCH) (18). Leptin appears to upregulate expression of hypothalamic neurotransmitters and neuropeptides that reduce appetite [eg. corticotrophin releasing hormone, POMC, cocaine- and amphetamine-related transcript (CART)], and downregulate expression of hypothalamic neurotransmitters and neuropeptides that increase appetite [NPY, agouti related peptide (AGRP)] (19,20). Two sets of neurons, one set co-expressing NPY and AGRP, and the other co-expressing POMC and CART, in close proximity to each other, are present in the arcuate nucleus. Axons from these project to the paraventricular nucleus and lateral hypothalamic area, two areas long known to modulate feeding behaviour (21).

Leptin has a dual regulation on energy balance. Under a steady state of energy homeostasis, it signals the level of adiposity, and under a non-steady state as in fasting and after a meal, it serves as a sensor independent of adiposity (10). In addition to central effects leptin also exerts a number of peripheral metabolic effects including increased expression of uncoupling proteins in the mitochondrial membrane thereby increasing energy expenditure (22).

A diabetogenic role for leptin has been suggested in view of hyperglycaemia and hyperinsulinaemia seen in ob/ob mice which respond to exogenous leptin (23). Children with insulin dependent diabetes mellitus have reduced leptin levels (5). There is a bi-directional regulation between the adipocyte and the endocrine pancreas involving leptin and insulin, and this is termed "adipoinsular" feedback

(24). Insulin stimulates synthesis and secretion of leptin from the adipocyte. Leptin in turn by acting directly on the pancreatic **0** cell and through the sympathetic nervous system via the hypothalamus inhibits insulin synthesis and secretion. Obesity associated diabetes mellitus appears to result from dysregulation of this adipoinsular feedback (24). High leptin levels themselves render target cells less responsive to leptin action. Hence desensitisation to leptin in the hypothalamus and in the pancreatic **P** cell will result respectively in hyperphagia and hyperinsulinaemia.

Much experimental evidence, including reversal of sterility in genetically leptin deficient ob/ob mice (IS) and advancement of sexual maturation in normal mice (25) in response to exogenous leptin administration, suggest that the well documented effects of nutritional status on reproductive function in humans are likely to be mediated by leptin. Genetic leptin deficiency (26) and leptin receptor defects (17) presenting with morbid obesity and hypogonadism occur in humans. Effects of leptin in modulating GnRH secretion are mediated by other hypothalamic neurons such as POMC and NPY. Direct effects of leptin at the pituitary (27) and ovarian (28) level have also been reported. The pubertal increase in leptin continues throughout all stages only in girls. In boys leptin levels begin to decline from Tanner stage 2, presumably as androgen secretion increases (5). Serum leptin levels vary according to the stage of the menstrual cycle (29) and altered leptin levels have been reported in polycystic ovarian syndrome and in anorexia nervosa, two reproductive dysfunctions that result from or are associated with metabolic and nutritional abnormalities (30).

Plasma leptin levels increase during pregnancy. Placenta and trophoblastic tissue become the major sources of leptin during pregnancy (9). Leptin levels return to nonpregnant values after childbirth. Cord blood leptin levels correlate positively with birth weight. Leptin is secreted in breast milk and is thought to regulate growth, promote haemopoiesis and lymphopoiesis, and function as a satiety factor in the newborn (30).

Evidence from two clinical settings, namely predisposition to osteoporosis in gonadal failure and protection against osteoporosis in obesity, prompted some investigators to look for a candidate hormone linked to both reproduction and obesity (31). Leptin was the obvious choice. Surprisingly, both genetically leptin deficient and leptin receptor deficient mice have a high bone mass despite gonadal failure and hypercortisolism, two conditions predisposing to osteoporosis. Even if obesity protected against bone resorption, a high bone mass would not occur in the presence of gonadal failure and hypercortisolism. Intracerebroventricular administration of leptin reduced the bone mass in ob/ob mice, and these effects are likely to be mediated *via* the hypothalamus. Downstream effectors of this pathway are yet to be ascertained.

Leptin was a hormone waiting to be discovered. Within seven years since its discovery extensive research established leptin as a major player in regulating a number of physiological processes. It does not act alone and its many partners in regulating body weight, reproduction, bone remodelling and other physiological processes are still being discovered. Leptin did not turn itself into a "magic bullet" against obesity in the way researchers, obesity sufferers and pharmaceutical companies expected in the wake of its discovery. Nevertheless it continues to provide fertile grounds for biomedical research.

References

1. Zhang Y, Proenea R, Maffei M, Barone M, Leopold L et al. Positional cloning of the mouse *obese* gene and its human homologue. *Nature* 1994; 372: 425-32.
2. Considine RV, Sinha MK, Heiman ML, Kriaucicunas A, Stephens TW et al. Serum immunoreactive-leptin concentrations in normal weight and obese humans. *New England Journal of Medicine* 1996; 334: 292-5.
3. Andrews JF. Leptin: energy regulation and beyond to a hormone with pan-physiological function. *Proceedings of the Nutrition Society* 1998; 57: 409-11.
4. Havel PJ, Kasim-Karakas S, Dubue GR, Mueller W, Phinney SD. Gender differences in plasma leptin concentrations. *Nature Medicine* 1996; 2: 949-50.
5. Blum WF, Englaro P, Attanasio AM, Kiess W, Rascher W. Human and clinical perspective on leptin. *Proceedings of the Nutrition Society* 1998; 57: 477-85.
6. Sinha MK, Ohannesian JP, Heiman ML, Kriauciunas A, Sytephens T W et al. Nocturnal rise of leptin in lean, obese and non-insulin dependent diabetes mellitus subjects. *Journal of Clinical Investigation* 1996; 97: 1344-7.
7. Sinha MK, Studs J, Ohannesian J, Magosin S, Stephens T et al. Ultradian oscillations of leptin secretion in humans. *Biochemical and Biophysical Research Communications* 1996; 228: 733-8.
8. Kolaczynski JW, Cosidine RV, Ohannesian J, Mareo C, Opentanova I et al. Responses of leptin to short-term fasting and refeeding in humans. *Diabetes* 1996; 45:1511-5.
9. Masuzaki H, Ogawa Y, Sagawa N, Hosoda K, Matsumoto T et al. Nonadipose tissue production of leptin: leptin as a novel placenta-derived hormone in humans. *Nature Medicine* 1997; 3:1029-33.
10. Caro JF, Sinha MK, Kolaczynski JW, Zhang PL, Considine RV. Leptin: the tale of an obesity gene. *Diabetes* 1996; 45:1455-62.
11. Wabitsch M, Jensen PB, Blum WF, Christofferson CT, Enlaro P et al. Insulin and Cortisol promote leptin production in cultured human fat cell. *Diabetes* 1996; 45: 1435-8.
12. Grunfeld C. Zhao C, Fuller J, Pollock A. Moser A et al. Endotoxin and cytokines induce expression of leptin, the *ob* gene product in hamsters. *Journal of Clinical Investigation* 1996; 97: 2152-7.
13. Taraglia LA. The leptin receptor. *Journal of Biological Chemistry* 1997; 272: 6093-6.
14. Campfield LA, Smith FJ, Guisez Y, Oevos R, Burn P. Recombinant mouse OB protein: Evidence for a peripheral signal linking adiposity and central neural networks. *Science* 1995; 269: 546-9.
15. Chehab FF, Lim ME, LUR. Correction of the sterility defect in homozygous obese female mice by treatment with human recombinant leptin. *Nature Genetics* 1996; 12: 318-20.

16. Montague CT, Farroqui IS, Whitehead JP, Soos MA, Rau H et al. Congenital leptin deficiency is associated with severe early-onset obesity in humans. *Nature* 1997; 387: 903-8.

17. Clement K, Vaisse C, Lahlou Z, Cabrol S, Pelloux V et al. A mutation in the human leptin receptor gene causing obesity and pituitary dysfuctions. *Nature* 1998; 392: 398-401.

18. Hakansson ML, Brown H, Ghilardi N, Skoda RC, Meister B. Leptin receptor immunoreactivity in chemically defined target neurons of the hypothalamus. *Journal of Neuroscience* 1988; 18: 559-72.

19. Friedman JM, Halaas JL, Leptin and the regulation of body weight in mammals. *Nature* 1998; 39S: 763-70.

20. Gura T. Tracing leptin's partners in regulating body weight. *Science* 2000; 287: 1738-40.

21. Schwartz MW, Woods SC, Porte D, Seeley RJ, Baskin DG. Central nervous system control of food intake. *Nature* 2000; 404: 661-71.

22. Lowell BB, Spiegelman BM. Towards a molecular understanding of adaptive thermogenesis. Nature 2000; 404: 652-60.

23. Harris RBS, Zhou J, Redmann SM, Smagin GN, Smith SR et al. A leptin dose-response study in obese (ob/ob) and lean (+/?) mice. *Endocrinology* 1998; 139: 8-19.

24. Kieffer TJ, Habener JF. The adipoinsular axis: effects of leptin on pancreatic-b cells. *American Journal of Physiology, Endocrinology and Metabolism* 2000; 278: E1-E14.

25. Ahima RS, Dushay J, Filer SN, Prabakaran D, Filer JS. Leptin accelerates the onset of puberty in normal female mice. *Journal of Clinical Investigation* 1997; 99: 391-5.

26. Strobel A, Issad T, Camoin L, Ozata M, Strosberg AD. A leptin missense mutation associated with hypogonadism and morbid obesity. *Nature Genetics* 1998; 18: 213-5.

27. Yu WH, Kimura M, Walczewska A, Karanth S, McCann SM. Role of leptin in hypothalamic-pituitary function. *Proceedings of the National Academy of Science USA*. 1997; 94: 1023-8.

28. Spicer LI. Fransisco CC. Adipose obese gene product, leptin, inhibits bovine thecal cells steroidogenesis. *Biology of Reproduction* 1998; 58: 207-12.

29. Hardie L, Trayhum P, Abramovich D, Fowler P. Circulating leptin in women: a longitudinal study in the menstrual cycle and during pregnancy: *Clinical Endocrinology* 1997; 47:101-6.

30. Mantzoros CS. The role of leptin in human obesity and disease: a review of current evidence. *Annals of Internal Medicine* 1999; 130: 651-7.

31. Amling M, Takeda S, Karsenty, G. A neuro (endo)crine regulation of bone remodeling. *BioEssays* 2000; 22: 970-5.

Kamani H Tennekoon, *Associate Professor in Physiology, Faculty of Medicine, University of Colombo, e-mail: khtennekoon@yahoo.com*

Miconazole

Possible interaction with warfarin prompts labelling changes

Miconazole, an antifungal agent, can be bought as an over the counter, non-prescription, vaginal cream or suppository. Reports with the U.S. FDA and the Canadian Adverse Drug Reaction database record that women using vaginal miconazole while on concomitant anticoagulant therapy such as warfarin have prolonged partial thromboplastin and prothrombin time. This has led Health Canada to ask manufacturers of vaginal miconazole products to add a new warning to the product monograph and to the product label. The warning will now state that those who are taking prescription anticoagulants such as warfarin should consult their physician or pharmacist before using vaginal miconazole, due to the risk of bleeding or bruising.

Reports in WHO-file: Miconazole and warfarin reported as interacting drugs

References

1. Canadian Adverse Reaction Newsletter 11:1, Jul 2001.

2. Health Canada Advisory, 15 Aug 2001. Available from URL: http://www.hc-sc.gc.ca