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Leptin and Undernutrition

Andrew M. Prentice, Ph.D., Sophie E. Moore, Ph.D., Andrew C. Collinson, M.D., and Maria A. O'Connell, Ph.D.

Introduction

The identification of leptin and the rapid elucidation of many aspects of its signaling pathways helped to fill in an important gap in the metabolic jigsaw.[1,2] Physiologists have long known that there must be a peripheral signal that can inform the central nervous system about the status of an organism's energy reserves, particularly its adipose tissue mass.[3,4] Coleman's parabiosis experiments in the 1970s proved this point, but the molecular mechanism had remained elusive for another 20 years.[5] Although leptin may not be the only such adipose-derived signal,[6] it is certainly a major player. Prior experience with hormonal signals such as insulin suggested that leptin would have pleiotropic actions in various end organs and that its signals would be integrated into a complicated system of checks and balances involving other hormones and neural pathways. These predictions have turned out to be true,[2] and the resultant complexities leave much yet to be discovered.[7]

The nature of the initial discovery of leptin (through its absence in a severe monogenic form of rodent obesity), together with its exceptionally high levels in obese people and the current obsession with potential pharmaceutical treatments for obesity, have all tended to label leptin as an "obesity hormone." The etymology of its name (leptos = slim, with the implied connotation of "slimming") compounds this problem and is rather unhelpful in trying to present a balanced perspective of its actions.[8] This review will take the opposite stance and examine the role of leptin in states of marginal nutrition or undernutrition of the type most likely to have driven metabolic selection processes during past millennia. Some of what follows expands on the initial thoughts of Jeffrey Flier in an article entitled "What's in a name? In search of leptin's physiologic role."[8]

Drs. Prentice, Moore, and Collison are with the MRC International Nutrition Group, London School of Hygiene & Tropical Medicine, 49-51 Bedford Square, London, WC1B 3DP and MRC Keneba, P.O. Box 273, Banjul, The Gambia, West Africa. Dr. O'Connell is with MRC Human Nutrition Research, Elsie Widdowson Laboratory, Fulborn Road, Cambridge, CB1 9NL, United Kingdom.

Leptin was at first assumed to be a satiety signal, and its failure to correct obesity in subjects lacking a genetic leptin defect was greeted with surprise and disappointment.[9,10] Most people still view leptin as a satiety signal first and foremost. However, a number of recent findings, combined with some more clear evolutionary-based arguments for the origins of leptin, force a re-examination of this mindset.[8,11] It appears likely that in obese people leptin's actions (and its perceived failures) represent aberrant behavior of a molecule removed from the circumstances it would have encountered throughout its evolutionary history. Indeed it can be argued that leptin might best be viewed as a "starvation hormone" whose major role is to signal an energy deficit rather than a surfeit.

The Evolutionary Origins of Leptin

Genetic mutations leading to a dysfunctional leptin molecule, or its receptor, produce a very marked phenotype with gross obesity and secondary infertility.[12–15] However, administration of human recombinant leptin to obese patients has little effect[11] except in those very rare cases of congenital leptin deficiency.[16] These and other findings suggest that leptin may be more conspicuous in its absence than in its presence. This raises a difficult paradox: How could evolutionary forces have created a molecule that functions best in its absence? Aspects of this conundrum will be discussed below, and some solutions suggested.

The regulation of food intake is often viewed as asymmetric on the grounds that avoidance of starvation carries a greater survival imperative than the avoidance of obesity. Darwin himself stated, "A grain in the balance will determine which individual shall live and which shall die."[17] Flier has extended this basic tenet and stated, ". . . it seems likely that a potent anti-obesity adipostatic system would be subject to negative genetic selection during the course of evolution."[8] The implication of this is that the leptin axis is not a potent anti-obesity system, but Flier's arguments may be flawed if taken to an extreme and if interpreted as indicating that leptin does not play a negative feedback role in body weight regulation.

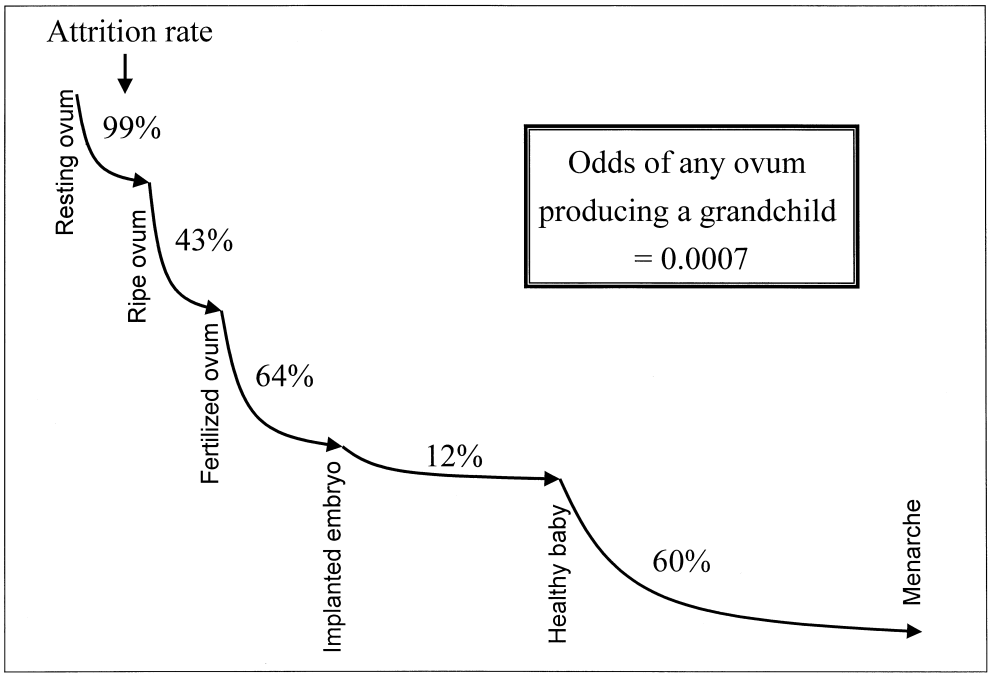

Figure 1. The timing of mortality contributing to natural selection in humans. Most evolutionary selection for fitness traits (e.g., a thrifty genotype) occurs prior to adulthood and reproduction. This may be significant in understanding the origins of the leptin axis. Reproduced with permission.[19]

The concept of a "thrifty genotype"[18] has been widely used to encapsulate the view that the ability to become fat when food resources allow it represents a positive selection trait and that the putative negative feedback role for leptin acts in opposition to this.[8] Flier argues that an effective role for leptin as an adipostatic hormone would subvert the thrifty genotype and would be predicted to reduce survival when food is scarce.

Elsewhere we have documented the fact that starvation has indeed been a major selective factor in human evolution even in quite recent generations.[19] However, it is probably incorrect to conclude that the selection of a thrifty genotype with an ability to lay down large amounts of fat is *necessarily* nature's solution to this challenge. Several other possibilities need to be considered.

First, the thrifty gene concept is usually envisaged as conferring a selective benefit to mature organisms that will be able to survive longer at times of famine. In fact, by far the greatest genetic selection occurs before the end of childhood, and most of this before birth (Figure 1). Thus the search for thrifty genes is most likely to bear fruit in relation to genetic traits that favor conception and fetal survival when nutritional conditions are harsh. JV Neel's initial ideas about thrifty genes where stimulated by his observations relating to the high birth weights of diabetic mothers.[18] Possible fetally expressed candidate genes for "thriftiness" (e.g., the insulin variable number of tandem repeats) are already emerging[20] and are under study by ourselves and others. Of course, maternal fatness is also a major determinant of fertility[21] and the more traditional view of the thrifty genotype (i.e., as conferring fatness) would accord with this fact.

The second caveat concerns the frequently made assumption that carrying extra energy reserves as fat is necessarily beneficial and carries no penalties. The issue of energy reserves needs to be examined over a series of different time scales. Acute food shortage reduces hepatic and muscle glycogen stores and threatens the organism's ability to fulfill its fight-or-flight reflexes. As a consequence there are multiple overlapping mechanisms to stimulate short-term hunger and food-seeking behaviors. In the short term there are few penalties to marginal overconsumption, and only gross overconsumption (leading to meal-induced torpor or lack of vigilance) would increase an animal's susceptibility to predation. Mechanisms exist to discourage such acute overeating. In the longer run, however, there are often penalties to carrying extra fat and in most species living in the wild body fat stores are finely tuned according to different environmental circumstances and the cycles of migration and reproduction.[22] For instance, the winter fattening of birds and mammals in high northern latitudes is not a simple consequence of changes in food availability between winter and summer, but is synchronously controlled by day length. Captive arctic birds resist fat gain until autumn and spontaneously shed fat in the spring even when fed an excess and constant ration throughout the year. Artificial manipulation of day length is a more powerful regulator of adipose tissue mass than are

changes in food supply or temperature. The role that leptin may play in controlling such changes is not yet known, but it is interesting that leptin's neural networks are intimately related to the melanocortin system.[2,23]

Many other examples could be raised to illustrate the fallacy of assuming that a feedback loop that down-regulates body fat would necessarily be the subject of negative selective pressure. Moving phylogenetically closer to humans it is clear that excess body fat is not a useful adaptation in arboreal primates whose primary defenses against predation are speed of movement and an ability to move to the thinnest branches where leopards fear to tread. Such primates maintain minimal fat stores in the wild. In humans the selective advantage of a lithe and quick physique to our hunting (and hunted) ancestors should not be underestimated. It might also be reasoned that in human childhood, which is abnormally extended according to allometric predictions,[24] there is a need for a negative growth modulator (i.e., appetite suppressant) to delay somatic and adipose tissue growth while brain development occurs.

Returning to the role of body fat in human reproduction there are also penalties to being excessively fat and the relationship between body fat and fertility shows a strong inverted U shape.[25]

Quite apart from the fact that there is no actual evidence in support of the thrifty gene concept there is a further flaw to the overly simplistic interpretation of the theory; namely, there are many ways to survive famine other than being fat.[19] Primo Levi's firsthand accounts of individual survival strategies in prisoners of war in Auschwitz described a range of personality traits (adaptability, guile, theft, work avoidance, determination, intelligence, and so forth) that favored survival in people whose fat stores were already minimal.[26] In other famine conditions the rich and powerful have the means to avoid starvation, and they frequently protect the beautiful, the clever, and the talented. Likewise, Fagan describes similar traits at the societal level in which, for instance, a willingness to migrate when local conditions deteriorate probably represented a survival mechanism as powerful as any metabolic adaptation.[27] This is especially so because metabolic thriftiness predicts an element of energy-sparing inactivity resulting in a fatalism that entraps the victims of starvation rather than offering an escape route.[28] In this respect it is also useful to recall that mass starvation has only become a feature in the post-agronomy era where natural catastrophes or warfare decimate crop supplies to large population masses.[27] Ancient warrior, hunter-gatherer, and nomadic races are less likely to have faced starvation and their descendents are therefore less likely to carry thrifty metabolic traits. This raises the possibility that thrifty mechanisms, and even some subtle aspects of the physiology of leptin, may map onto different racial characteristics.

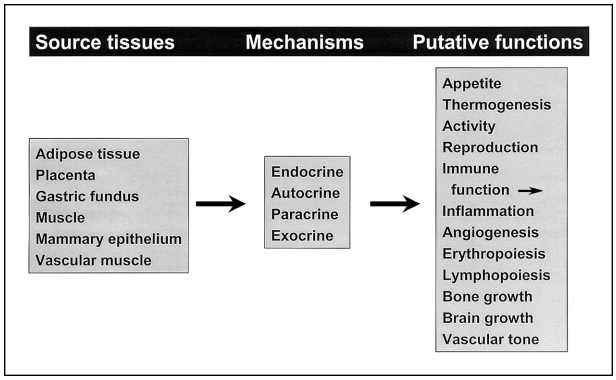

Figure 2. The pleiotropic effects of leptin.

The purpose of this short anthropologic detour was to make the point that we cannot assume that an adipostatic role for leptin would have necessarily been subject to negative genetic selection. Rather precise regulation of body fat is the norm in almost all vertebrates living in the wild and the current grossly aberrant dysregulation seen in humans may misinform theoretic constructs concerning leptin's evolutionary origins.

It is also worth noting that many of leptin's actions may be somewhat vestigial in humans. In primitive evolution the earliest signaling molecules and their receptors would have been very simple molecules whose complexity gradually evolved both in terms of their molecular nature and their function. Concerning ourselves with the paradox of how leptin evolved when it is most important in its absence may be missing the point that it initially evolved for quite different purposes than those to which it has now been adapted. It is possible that humans are currently in a phase of reductive evolution in which the phylogenetic adipostatic functions of leptin have become somewhat redundant in the absence of predation (and that may suddenly be the subject of positive selection again following the abrupt ecologic transition in food and activity at the end of the 20th century). This argument that leptin is vestigial could be applied separately to the various different actions of leptin—some may be vestigial, others not.

The Pleiotropic Actions of Leptin

In the few years since its initial discovery leptin has had numerous functions ascribed to it as summarized in Figure 2. This list will no doubt expand further. The experimental evidence is derived from a number of sources and some of the claimed functions may turn out to be false associations demonstrable under certain conditions of cell culture, but of little significance in real life. Nonetheless it is necessary to bear this list in mind

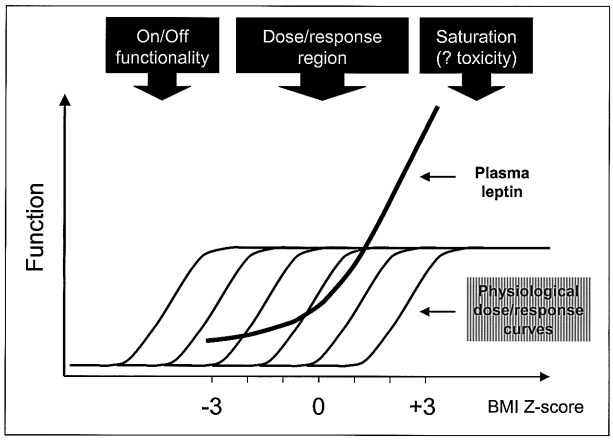

Figure 3. Schematic representation of dose-response curves linking circulating leptin to functional outcomes. Different organs, or even different cell types within organs, may exhibit different dose-response sensitivities to leptin and these may in turn be shifted to the right or the left as part of metabolic adaptive processes. BMI = body mass index (kg/m^2).

when considering the likely role of leptin in orchestrating physiologic responses to undernutrition.

Dose-Response Characteristics of Leptin

When evolution has pressed a single molecular species such has leptin into a number of different functions in several organs there must be mechanisms to permit variable sensitivity across the physiologic range of circulating leptin levels. Nature achieves this through a number of strategies including filtering the level of hypothalamic exposure by means of the blood-brain barrier (see below), anatomic variations in receptor expression, variations in receptor subtypes, up- and down-regulation of cell surface expression of receptors, membrane modulation of receptor sensitivity, the actions of competitive binding proteins, and neural modulation of postreceptor actions. This allows for a wide range in the dose-response relationships between leptin concentration and its physiologic action as illustrated in Figure 3. The existence of a spectrum of dose-response curves would allow leptin to show on/off functionality in certain respects, sensitive dose-response relationships in other respects, and functional saturation (often termed "leptin resistance") in yet other respects. Not only are these curves likely to be different for each of leptin's actions, but also they are likely to be subject to long-term modulation as the organism becomes gradually accommodated to different planes of energy flux and leptin levels. For example, Ozata and colleagues described spontaneous compensation for some of the effects of leptin deficiency on immunity and endocrine functions.[13]

These likely variations in dose-response curves are further complicated by the issue of acute versus tonic regulation. In engineering control systems designed to maintain homeostasis, a constant tonic signal is frequently less useful as a regulatory input than an acute change in signal. Evidence indicates that this is likely to be the case for leptin because it responds to relatively short-term changes in energy balance in a manner quite disproportionate to the associated changes in fat mass.[29,30] The time scale for such changes varies from approximately 12 hours to days and weeks. It appears that leptin is not involved in shorter-term regulation because it does not display any major postprandial changes like insulin.[31]

Examples of the medium-term modulation of circulating leptin levels can be seen in response to both positive and negative deviations of energy balance.[29,30] For instance, men on a weight-reducing diet (21% weight loss, 28% fat loss over a mean of 95 days) showed a 76% reduction in plasma leptin levels.[31] The change in plasma leptin was therefore magnifying the change in fat loss. This may be due to the fact that the subjects were still in a physiologically perceived state of energy deprivation after weight loss. The same authors described a similar decrease in plasma leptin of approximately 75% in response to just a 3-day fast accompanied by a 1.7-kg weight loss, thus emphasizing the extent to which leptin is able to send a magnified signal to the hypothalamus in response to short-term energy deprivation.[31] Numerous other studies have replicated this finding. In the reverse direction of energy excess we have performed a study of progressive overfeeding with 20, 40, and 60% energy excess over 3-week periods with 1-week periods of free feeding in between.[32] Plasma leptin increased by 34, 83, and 142% by the end of each period of excess in spite of body fat gains of only 3, 8, and 37%. After a further 3-week ad libitum period at the end of the entire experiment, body fat was raised by 20% and plasma leptin by 17%. These results clearly illustrate that leptin provides readout of both the dynamic and the chronic state of energy stores. When energy balance is perturbed the dynamic component greatly outweighs the static component.

In the absence of these short-term modulations in response to acute variations in energy balance leptin provides a remarkably reliable measure of adipose tissue volume (with separate relationships in males and females), and appears to remain virtually linear even at very high[2,33] and at very low[34–36] levels of body fat. Figure 4 superimposes data from three studies of anorexic patients[36] onto a regression line derived from a small group of individuals studied in our own laboratory. The anorexic patients display leptin levels that seem to lie on the expected prediction line.

However, this exquisitely accurate linkage between body energy stores and circulating messenger does not necessarily translate into a proportionate readout at the

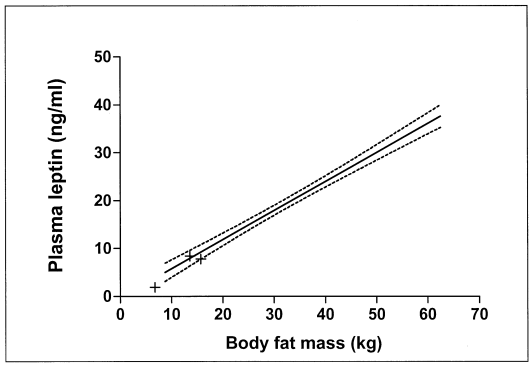

Figure 4. Leptin levels in anorexia nervosa. Reference curve (with SEE) derived from a small group of normal subjects (Murgatroyd & Prentice, unpublished, 2001). Crosses indicate patient data from Polito et al.[36]

hypothalamus. One of the earliest published studies on leptin demonstrated a markedly reduced cerebrospinal fluid (CSF)–to–plasma leptin ratio in obese subjects and argued that this was the cause of leptin resistance.[37] In severe undernutrition the reverse occurs with a proportionately greater CSF-to-plasma ratio as illustrated in Figure 5. This may indicate the presence of a saturable transport process across the blood-brain barrier, and has the effect of attenuating the slope of leptin's response to altered energy balance. Whether this attenuation represents a long-term tonic accommodation phenomenon or occurs in response to short-term changes is not yet known. Likewise the functional origins of these changes are not understood and would appear to undermine the potency of the afferent signal coming from energy reserves. One possible explanation of this could be that other organs require a more magnified readout of energy reserves than does the brain.

Leptin as a Starvation Hormone: Functionality at Low Levels

As discussed above, the fact that obese people have very high levels of plasma leptin tends to contradict any theory of a simple feedback loop in which leptin is a satiety signal that can effect a down-regulation of food intake and an up-regulation of thermogenesis. However, when recombinant leptin is injected into constitutionally deficient *ob/ob* mice, these are precisely the effects that are observed.[39,40] Even in wild-type mice exogenous leptin has similar effects though the magnitude is greatly attenuated.[39,40] Similar results are seen in humans. Daily injections of human recombinant leptin to the world's first-discovered leptin-deficient patient caused a remarkable decrease in appetite and rapid and sustained weight loss.[16] Interestingly there was no detectable change in energy expenditure once corrected for the changes in body composition, which emphasizes the species differences in leptin's actions. The weight loss in this patient occurred in spite of the fact that the average diurnal circulating leptin concentration achieved by the recombinant product was only a fraction of the level predicted on the basis of the patient's fat mass.[16] This observation parallels the findings from the early parabiosis experiments in which only a small amount of blood exchange between the parabiosed animals was sufficient to have a pronounced effect on weight loss in the *ob* member of the pair.[5] Thus leptin-naïve animals are especially sensitive even to low levels of leptin. This has led to suggestions of an "on/off" functionality and is the origin of the paradox suggesting that the hormone may be more important in its absence than its presence.

By contrast, a large clinical trial using exogenous dosing with human recombinant leptin in obese subjects lacking a constitutive leptin defect yielded minimal weight loss.[11] Together with the general observation of high leptin levels in obesity this has encouraged the frequent use of the phrase "leptin resistance." This phrase carries an unfortunate implication of an active development of resistance and of a failure in physiologic functioning. Reference to the dose-response curves in Figure 3 indicates that leptin may never have evolved to

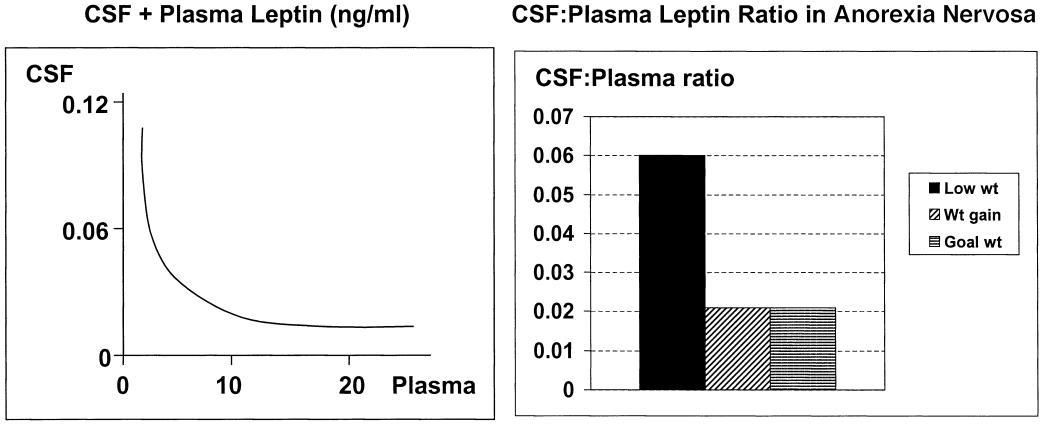

Figure 5. Alterations in CSF:plasma leptin ratios in anorexia nervosa. CSF = cerebrospinal fluid, Low wt = anorexic state, wt gain = during recovery, goal wt = at target recovery weight. Redrawn from Mantzoros et al.[38]

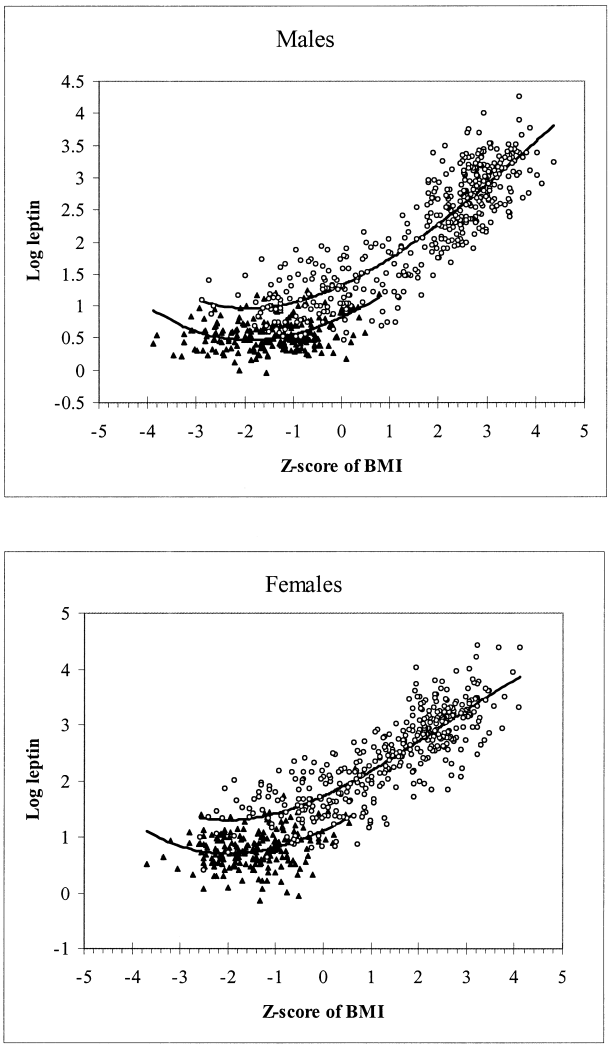

Figure 6. Comparison of plasma leptin levels in Italian and Gambian children. Data from children aged 6.5 to 9.5 years. Open circles = Italian, triangles = Gambian. Individual BMIs (kg/m^2) are expressed as Z-scores relative to United Kingdom standard growth curves. Reproduced with permission.[41]

function at such high levels and that the term leptin resistance is therefore misleading and unhelpful.

In summary, it can be concluded from the above that leptin has probably evolved to act most potently at low levels of energy sufficiency and may be particularly important in signaling across the transition between energy sufficiency and insufficiency. Figure 6 emphasizes the importance of focusing on these low levels of body fat (and resultant low levels of leptin) when considering leptin's natural biology and the likely conditions under which it evolved. It illustrates a comparison of leptin levels in undernourished Gambian children compared with an age- and sex-matched reference group from Italy.[41] Leptin levels show quite a shallow slope against body mass index (BMI, kg/m^2) for all individuals below the reference norm (i.e., zero standard deviation score). Most of the Italian children were above the reference norm indicating a chronic energy surplus that must be stored as fat. Under these conditions leptin rises steeply and reaches levels that would have been supraphysiologic over most of evolutionary time. It may therefore be unreasonable to try to make teleologic interpretations of function, and presumed dysfunction, of the leptin axis in most Western individuals. Focusing on the more natural states of energy deficit, Figure 6 indicates an offset between the Gambian and Italian children. There may be several explanations for this. One is that the lean-to-fat tissue ratio may differ between the two groups with Africans being proportionately leaner at any given BMI.[42] A second is that thin Italians may be constitutionally lean even though perfectly well nourished, whereas the leanness of the Gambians genuinely represents a chronic dietary energy deficit. This latter explanation would fit with the fact that leptin is a sensor of both adipose tissue mass and acute energy balance. In line with this interpretation is the fact that in younger infants in The Gambia the seasonal swings in leptin between the hungry and harvest seasons are greater than the changes in body mass, again emphasizing the acute-on-chronic nature of the leptin readout of energy status (AC Collinson, unpublished results, 2002).

Having set the scene with these general remarks about the function of leptin in undernutrition, the remainder of this review will briefly summarize aspects of knowledge in certain key physiologic states where malnutrition threatens the survival of the individual and hence their genetic lineage.

Leptin and Thyroid Function: Orchestrating Adaptive Responses to Starvation

Flier and colleagues have emphasized the need to move away from the common clinical view of thyroid physiology based on static levels providing tonic regulation of metabolic homeostasis.[43] In fact, thyroid hormones play a major role in orchestrating the medium and long-term adaptations to starvation with decreases in T3 signaling, a suppression of basal metabolic rate, and a reduction in protein turnover and nitrogen loss.[44] The time course of these changes differs greatly between species according to their susceptibility to starvation (small animals are very susceptible owing to their relatively high metabolic turnover rate), but the underlying processes appear similar.

Recent findings suggest that leptin is the dominant signal to the brain that triggers a reduction in thyroid-releasing hormone (TRH) in the paraventricular nucleus.[43] Although the ObRb isoform of the leptin receptor has been found in TRH neurons it appears likely that falling leptin achieves its effect indirectly (at least in part) through leptin-responsive pathways communicating with the paraventricular nucleus (PVN) because chemical ablation of the arcuate nucleus blocked the normal

hypothyroid response to starvation.[45] Subsequent experiments have revealed that in rodents the neural pathway from the arcuate nucleus to the PVN involves Agouti-related protein, which is suppressed by leptin, and alpha melanocyte–stimulating hormone, which is induced by leptin.[43,46] Thus the central melanocortin system can mediate the actions of leptin on thyroid function. The interplay of these systems in human physiology have not yet been explored in detail but it is likely that leptin will emerge as a major coordinating signal for the hypothalamic-pituitary-thyroid axis during the transition from the fed to the starved state.

Leptin and the Reproductive Axis

The role of leptin in regulating the reproductive axis has been reviewed in detail elsewhere in this supplement[47] so we confine ourselves here to a few observations in specific reference to undernutrition in humans. Forty years ago, Rose Frisch developed her theories relating the onset and maintenance of reproductive function in women to a critical minimum level of body fat.[21] She combined a series of observational studies in gymnasts, athletes, anorexic patients, and deprived populations to generate a threshold level of body weight and fat below which the reproductive axis is suppressed. This represents a logical protective strategy for reproductive scheduling that allows the mother to defend her own survival until better times arrive and she can reproduce successfully. Leptin provides an ideal candidate for the peripheral signal inferred in Frisch's theory and, in its absence, certainly blocks reproduction.[47] Leptin levels also appear to predict the age of menarche in girls[47] but whether this is a causal link is not yet certain.

Over-interpretation of Frisch's thresholds (by both herself and others) tended to undermine her elegant thesis because, for instance, most women in the Indian subcontinent were reproducing at body weights below the claimed thresholds. This emphasizes the need to envisage complex control processes in which the role of leptin is to report both static and dynamic levels of energy status, and in which its signaling pathways are subjected to a range of other neural inputs that can act as agonists or antagonists. Initial failures to detect simple relationships between circulating leptin levels and function do not necessarily imply that leptin is not playing a central role.

Maternal Metabolic Responses to Undernutrition in Pregnancy: A Signaling Role for Leptin?

Detailed prospective studies of the energetic adaptations of human pregnancy performed in our own and other laboratories have shown a high level of metabolic plasticity that allows women on a low plane of nutrition to achieve a full pregnancy with a much lower energy cost than better-nourished women (Figure 7).[48–50] This represents a powerful protective mechanism that will have been the subject of strong selective pressures because it ensures fetal well-being in marginal conditions. Prior to the discovery of leptin we speculated that there must be a peripheral signal capable of detecting the state of long-term energy reserves (adipose tissue mass) and short-term energy status (positive or negative fluxes). Leptin provides a likely candidate molecule that would meet these requirements. Unfortunately the studies summarized in Figure 7 have no residual plasma samples that could be analyzed for leptin. In any case, interpretation of such results would be very complex given the long time course of gestation and the dual role of leptin in signaling an integrated estimate of chronic and acute energy status. Furthermore experimental animal studies cannot help with this particular question because the incremental energy needs required to support large and fast-growing litters are so great that there would have been no selective pressure in favor of marginal energy-sparing adaptations of the type seen in humans.[51] New human experiments will therefore be required to test the prediction that leptin plays a central role in coordinating adaptations to pregnancy.

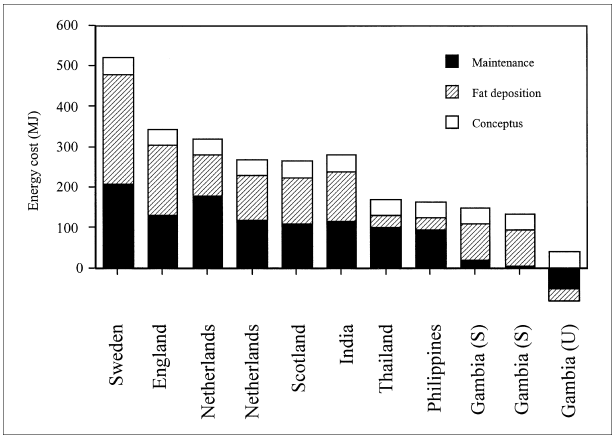

Figure 7. Plasticity of energy requirements in pregnancy in populations on different planes of nutrition. Bars represent cumulative estimates of the energy stored as products of conception (labeled conceptus), body fat, and energy expended on maintaining the pregnancy. Data were calculated using estimates made in the prepregnant state and at 6 weekly intervals throughout gestation. Reproduced with permission.[50]

Leptin and the Fetus

Leptin is produced by both the fetus and the placenta.[52] Placental leptin is secreted into both the maternal and fetal bloodstreams, but at a much higher level on the maternal side. These observations suggest that leptin plays an important role in the integration of maternal and

fetal physiology during gestation, and one possible example of this has been suggested above.

In humans fetal blood leptin levels (which may originate from a mixture of fetal or placental origin) are extremely low until approximately 34 weeks gestation when they start to rise sharply until term.[53] Preterm babies therefore have much lower leptin levels at birth compared with term babies (e.g., 4.6 ± 6.9 vs. 19.6 ± 14.3 ng/mL, respectively[53]). Leptin levels show a precipitate fall in the newborn suggesting that much of the fetal leptin is placentally derived.[54]

At birth cord blood leptin is strongly correlated with birth weight, ponderal index, length, and head circumference,[55] and thus babies suffering intrauterine growth retardation (IUGR) are also characterized by low leptin levels.[38] It is not yet known whether this represents a causal link or simply a reflection of the fact that IUGR babies have less fat.

Leptin is conditionally essential for conception (see above) and, because leptin receptor mRNA can be found in several murine fetal tissues, it is proposed to play a role in fetal maturation.[52] Two lines of evidence suggest that once conception has been achieved, however, leptin is no longer essential for the maintenance of subsequent fetal growth and well-being. First, after leptin administration to aid conception, leptin withdrawal from *ob/ob* dams at different stages of pregnancy did not affect pregnancy rate or litter size in mice (though the dams did not lactate).[56] Second, although no homozygous leptin- or leptin receptor–deficient humans have been studied in detail at birth, all known individuals appear to have been relatively normal (at least in terms of size) on the basis of clinical history.[12,14,15]

The role of leptin in the immediate postpartum period and the significance of the precipitate drop after parturition are not fully understood but are likely to be involved in aiding the transition from fetus to neonate in terms of altered fuel selection and non-shivering thermogenesis.[57]

Leptin and the Control of Lactation

Lactation represents the most anabolic period of adult life when women need to increase their energy intake by approximately 25%.[58] Milk yield is subtly controlled so that the rate of synthesis matches the infant's requirements. The infant's demand is transmitted to the mother through the frequency, duration, and intensity of suckling, all of which modulate oxytocin and prolactin release. Because leptin is known to modulate appetite (on the infant side) and might modulate lipolysis (on the maternal side) we could speculate an important role in lactation.

Ob/ob mice are unable to lactate without exogenous leptin,[56] but the possible effects of low (as opposed to absent) leptin in humans appear not to have been studied yet. Our previous work in this field shows that human lactation is surprisingly robust in the face of maternal leanness and energy deficiency.[59] There appears to be no detectable effect of maternal thinness on milk volume even down as low as maternal BMI of 16.5 or less, implying that very low leptin levels are permissive of adequate lactation.

From the child's perspective, humans are notable for their very slow physical development in childhood and are the only species to have a true pubertal period of re-accelerated growth leading to sexual maturity.[24] Many investigators have argued that the slow growth is an adaptation that allows time for the development and training of our large brain. Growth is regulated by complex processes involving, among others, the insulin-like growth factors (IGF), their binding proteins, growth hormone, and the sex hormones. These generally act as positive drivers of growth. The uncontrolled growth of leptin-deficient children demonstrates that leptin plays the opposite role by down-regulating food intake and ensuring that somatic growth (especially of adipose tissue) does not run ahead of the slowly maturing brain. Might this be one of the most important functions of leptin?

Leptin may also play a role in mediating the duration of postpartum infertility. This is chiefly achieved through a sustained lactational amenhorrea, which in turn is controlled by raised prolactin.[60] Prolactin levels are strongly influenced by the intensity and frequency of infant sucking.[60] However, our own studies of seasonal variations in plasma prolactin levels in lactating Gambian women and of the impact of dietary supplementation confirm that the mother's energy status is also crucial.[61] Once again leptin is an ideal candidate as an intermediary signal. Evidence from rodents (where the energy stress of lactation is manyfold higher relative to the mother's metabolic body size than in humans) suggests that decreases in leptin caused by the energy drain of lactation are closely related to the suppression of pulsatile leutinizing hormone secretion and reproductive function but are not necessarily a prerequisite.[62,63] In humans the appropriate studies in marginally nourished women have not yet been done, and data from well-nourished women are variable, probably as a consequence of the fact that they are free to meet the energy needs of lactation through increased food intake, utilization of body fat, or a combination of the two. The best-controlled study showed 25% lower circulating leptin levels in lactating compared with nonlactating women at 3 and 6 months postpartum, but the differences were not significant.[64]

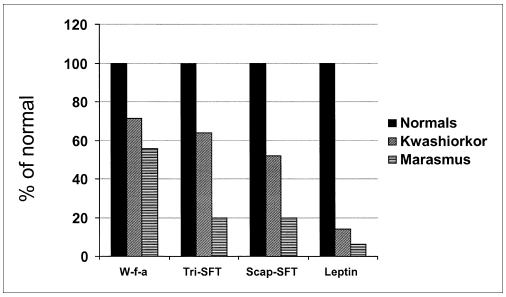

Figure 8. Leptin levels in severe protein-energy malnutrition (PEM). Data from 20 children with marasmus, 10 with kwashiorkor, and 10 age-matched normal children. W-f-a = weight-for-age, Tri-SFT = triceps skinfold, Scap-SFT = subscapular skinfold. Redrawn from reference 66.

Leptin in Severe Protein-Energy Malnutrition

Despite many theories, the reasons why severe childhood malnutrition displays the two distinct clinical syndromes of marasmus and kwashiorkor remain unresolved. Marasmus is classically viewed as arising from balanced protein and energy deficiency and is considered an adapted state in which muscle breakdown is used to maintain relatively normal plasma albumin levels. Kwashiorkor is thought to represent a relative energy sufficiency with unbalanced protein deficiency. It is viewed as a maladapted state and is associated with low levels of plasma albumin. It has been proposed that variations in the ratios of insulin, cortisol, and growth hormone are implicated.[65] Might it be possible that a relatively higher fat mass, and hence leptin, in the pre-kwashiorkor state could lead to the maladapted state by suppressing cortisol and growth hormone?

Data from children with severe protein-energy malnutrition are illustrated in Figures 8 and 9.[66] Figure 8 reveals that triceps and subscapular skinfold thicknesses are greater in kwashiorkor than in marasmus. This is reflected in significantly higher leptin levels (0.95 ± 0.20 SD vs. 0.41 ± 0.18 ng/mL, respectively, $P < 0.05$), but both of these are much lower than in normally nourished controls (6.34 ± 1.10 ng/mL). Figure 9 shows that there are differences in insulin, IGF-1, and peak growth hormone between marasmus and kwashiorkor, but the study does not allow any causal interpretations between leptin and these other hormones or between these and the clinical form of the malnutrition.

Leptin and Immune Function in Undernutrition

Evolution has equipped most living organisms with a series of adaptive mechanisms by which they can prolong their survival when nutrient supply is suboptimal. These adaptations are controlled processes involving apoptotic shutdown and organ hypotrophy that can delay the time at which catastrophic collapse would occur. In the early stages of malnutrition there is scope for tightening the efficiency of various metabolic functions and for closing down some that are nonessential in the short term (e.g., reproduction). If the situation progresses toward severe malnutrition the organism must make much more radical "decisions" and take a series of gambles (the efficacy of which has been tested through thousands of previous famines).[19] A gradual down-grading of immune function might be one such strategy, but in order to maximize the odds of survival this needs to be carefully calibrated against one or more measures of nutritional status. Leptin is an obvious candidate and could operate on different aspects of immune function at different degrees of malnutrition (Figure 3).

There remains considerable controversy about the extent to which human immunity is compromised by mild-to-moderate malnutrition. On the one hand there are many reports of impaired function across a range of multiple and single deficiencies,[67] and on the other it is argued that cross-sectional associations between malnutrition and immunodeficiency can be ascribed to the concomitant presence of infection as a cause rather than as an effect of malnutrition.[68] From a teleologic standpoint, the degradation of many innate protective mechanisms (e.g., barrier defenses including epithelial integrity, secretory IgA, lactoferrin, lysozyme, etc.) could be immediately fatal by permitting the entry of enteric and other organisms that are normally harmless commensal bacteria. Furthermore these processes probably have a *relatively* low energy demand. It is more likely that cognate immunity may be suppressed for two reasons. First, that the maintenance and propagation of a large circulating pool of leukocytes has a high energy demand. Second, that in much of our evolutionary past we existed as small hunter-gatherer groups that rarely came across other groups capable of spreading novel human pathogens. Thus, although cell-mediated memory-based defenses are optimal for survival, they are not always necessary and could probably be suppressed in times of extreme nutritional need in order to spare energy for processes of more immediate survival value. This line of

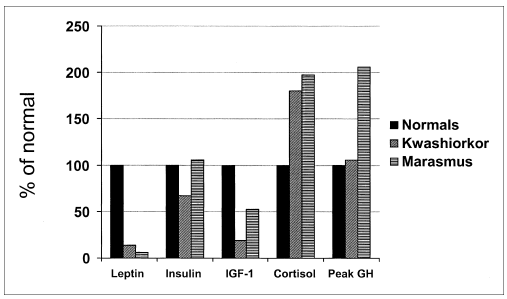

Figure 9. Hormonal profiles in severe protein-energy malnutrition. Data from 20 children with marasmus, 10 with kwashiorkor, and 10 age-matched normal children. IGF-1 = insulin-like growth factor 1, GH = growth hormone. Redrawn from reference 66.

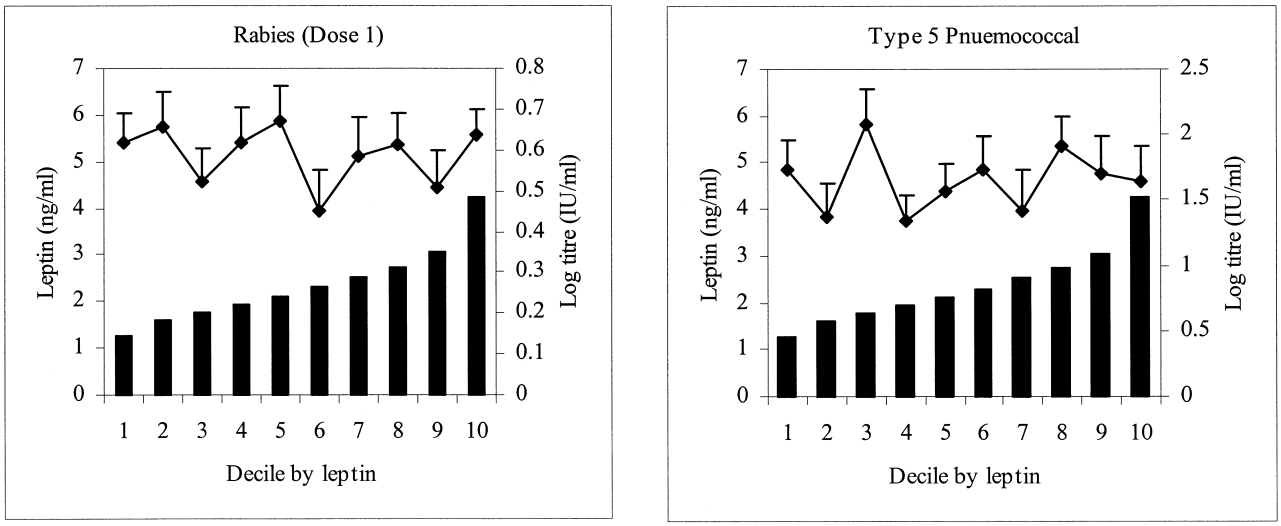

Figure 10. Lack of relationship between leptin and measures of immune function in Gambian children. Data from 472 children aged 6.5 to 9.5 years with body mass index Z-scores ranging from +0.9 to −4.7.[73] Rabies and pneumococcal responses assessed as quantitative seroconversion. Subjects were classified by decile of plasma leptin level.

argument helps to predict where leptin is most likely, if at all, to affect immunity. It turns out that the evidence so far accrued in animals suggests that it is cell-mediated immunity that is the likely target of leptin's regulatory influence.

T-cells express the active form of the leptin receptor[69,70] and an elegant series of experiments has demonstrated that leptin stimulates T-cell proliferation in culture for wild-type mice but not for *db/db* (receptor-deficient) mice.[70,71] *Ob/ob* mice also display some signs of immunodeficiency, though this is not very marked.[70,71] A second set of experiments showed that leptin pretreatment completely protected mice from starvation-induced thymic atrophy and from the consequent marked reduction in T-cell output,[71] suggesting that leptin is the key signal of starvation that prompts the well-known phenomenon of "nutritional thymectomy."[72] Lord has summarized these and other experiments in detail in an accompanying paper in this volume.[70]

The extent to which low leptin levels affect immune function in humans in the real world is still open to question. Ozata et al.[13] have published an analysis of survival in a leptin-deficient Turkish family. Based on verbal autopsies they recorded pre-adult mortality rates as follows: heterozygotes 0/19, homozygotes 7/11, Chi-squared = 25.4, $P < 0.01$. Clearly this data can only be viewed as preliminary in view of the method of its collection.

We have studied the possible relationship between leptin and immune function using vaccine responses (23 valent pnuemococcal capsular polysaccharide vaccine, Pneumovax®, and the Human Diploid Cell Rabies vaccine) in a large group of Gambian children suffering chronic moderate-to–quite severe malnutrition.[73] Studying vaccine responses provides an ethical means of assessing the integrity of the full sequence of events from antigen recognition to immunoglobulin production. Figure 10 illustrates that there is no detectable association between deciles of leptin and the responses to either the pneumococcal vaccine (primarily B-cell driven) or the rabies vaccine (primarily T-cell driven). Similarly there was no association with the CMI tests. These results do not rule out an effect of leptin at more severe states of malnutrition, but it should be noted that the children in the lowest quartile had an average weight-for-age Z-score of −2.8 and had very low leptin levels compared with European children (see Figure 6 derived from the same group of children).

There is much still to be learned about a possible modulatory role for leptin in relation to immune function, but our preliminary human data suggest that if a biologically significant role does exist then it is likely to occur only at very extreme levels of energy (and leptin) deficiency.

Conclusion

Further investigations are required to elucidate the possible modulatory role of leptin in states both of energy deficit and excess, but it is abundantly clear that it is a major integrator of peripheral and central processes interacting in complex ways with other hormonal and neuronal axes. Teleologic reasoning based on the likely evolutionary origins of the molecule suggest that many of its most important functions will occur at low levels of energy status, and in coordinating the metabolic transition between the fed and the fasted state. Attempts to understand its function through the studies of overnour-

ished and obese people that now dominate the literature may be highly misleading, as may be the term "leptin resistance." The apparent evolutionary paradox raised by the suggestion that leptin is more important in its absence than in its presence represents an exaggeration of the existing data and can be easily resolved if the functions of leptin have transmuted over time and with species division and development. A greater future emphasis on studying leptin in undernourished people would add to the rapidly developing state of knowledge on leptin and may resolve some of the existing controversies.

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