Leptin: Defining Its Role in Humans by the Clinical Study of Genetic Disorders

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Extremely unusual genetic conditions can reveal normal processes governing physiologic regulation and metabolism. Children with rare homozygous mutations in the leptin gene and complete leptin deficiency develop extreme hyperphagia and obesity soon after birth but respond with normal eating and a selective loss of excess body fat upon being given small amounts of leptin. Heterozygote relatives have 30% more fat than predicted and relatively low leptin levels. This demonstrates leptin's fundamental involvement in maintaining energy balance. Leptin also seems to act as a metabolic gate allowing children to enter puberty.

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The value of careful studies of individuals with major genetic abnormalities can often illuminate the normal physiology that underlies complex conditions such as the maintenance of body weight. The studies on the control of leptin production, circulating leptin, receptor activity, and the neurochemical pathways involving leptin, e.g., the generation of pro-opiomelanocortin (POMC) and the integrity of the melanocortin 4 (MC4) receptor, are remarkably illuminating. Homozygous mutations in the genes relating to leptin and POMC production or receptor function are associated with extremely severe obesity of childhood onset. Human studies suggest some differences in neuroendocrine networking from that seen in experimental animals, and more detailed analyses now reveal that there are more subtle changes in circulating leptin and its relationship to body weight regulation in heterozygous individuals. The onset of puberty, the regulation of bone maturation, and the modulation of insulin and glucose metabolism are all included in the array of actions being modulated by changes in circulating leptin and the subsequent activation of the neurochemical control of the hypothalamic pituitary adrenal axis.

The study of extreme human variation can often provide a very valuable tool in the understanding of human physiology and pathophysiology. If one just considers the recent endocrinologic literature this concept of using extreme conditions to reveal normal biologic control mechanisms is well displayed. Thus the study of a couple of very tall people revealed for the first time that estrogens are an absolutely critical regulator of the fusion of bone and the finalization of both maturation and growth during adolescence.[1] Conversely, the study of a very short individual by Woods et al.[2] found that the associated mental retardation was almost certainly related to a lack of insulin-like growth factor 1 (IGF-1) activity. This placed IGF-1 as a critical regulator of brain growth and development in humans. The work of Lifton[3] has also beautifully demonstrated how human genetic disorders can provide novel information about the central role of the kidney in regulating blood pressure. Clinicians live in a natural laboratory created by their patients. We have attempted over the last 9 years to exploit this natural laboratory and have focused on two areas: extreme obesity or extreme insulin resistance. This review focuses on the obesity studies.

There are several reasons for studying these extreme phenotypes. One is the humanitarian need. The affected individuals are in extreme distress and suffer serious morbidity and an early death. The second reason is the illumination of normal biology where often we find striking parallels with the biology of lower mammals. Sometimes, however, new biologic aspects emerge and act as markers that help us to think about the more common disorders affecting mankind. Dr. Sadaf Farooqi and I have, over the last few years, been looking at a cohort of patients with genetically determined obesity. These patients have been referred from all over the United Kingdom, Europe, and indeed the Middle East. We have now accrued approximately 550 unrelated individual children with severe obesity in the prepubertal phase, i.e., with obesity starting before the age of 10

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years. These individuals have a mean body mass index (BMI, kg/m^2) that is 4.5 standard deviation scores above the median BMI for children of the same age and sex. So they are really an extraordinarily extreme group of children whose mean age of onset of obesity is less than 5 years. The first two children that forced us to think in a new way were first cousins born to parents of Pakistani origin living in the United Kingdom. One girl aged 9 was 96 kg and had been nearly 30 kg at 2.5 years.[4] Both were born of normal weight but within a few months they were severely obese, one child had liposuction of her thighs at the age of 2 to allow her to walk and by the age of 4 she was wheelchair bound. The second, younger child was headed in the same direction. Behaviorally and developmentally they were essentially normal except for their intense hyperphagia. They were always hungry and demanding food and were very unhappy if food was not provided. For example they would raid the freezer at night if the refrigerator was locked and would eat frozen food even before it was thawed. They had therefore intense and very abnormal hyperphagia that was exceptionally difficult to manage for both the parents and for the older child once she began to attend school. These children proved to have undetectable plasma leptin levels, despite massive obesity. They had a frame-shift mutation in the leptin gene involving the loss of the last 34 amino acids in leptin including the C terminal cysteine essential for biologic activity.[5]

Both children were homozygous for this mutation and their three wild-type and one heterozygous siblings were lean. In practice it is now clear that the homozygous abnormality is rare because in approximately 450 unrelated children with extreme early-onset obesity in whom we have measured leptin, we have found only two further families, one from the United Kingdom and another from Canada. These two other families were also of Pakistani origin; the affected children were the offspring of consanguineous parents and had an identical mutation. The parents' families were not closely related over the last four or five generations. This means that this may be an ancestral mutation in people of Pakistani origin, or else that this region of the leptin gene, which contains a run of six G nucleotides, is prone to instability. We have not found any adults yet with this leptin gene mutation but it is noteworthy that Strobel et al.[6] described consanguineous Turkish kindred with a homozygous mutation. It is striking that two of the individuals who could be studied in detail had clear evidence of hypogonadism and did not enter puberty until well into their 20s and 30s. This provides clear evidence that leptin was necessary for humans to enter puberty. Then Clement et al.[7] found three humans who completely lacked leptin receptors. This confirmed the importance of the leptin receptor in terms of human appetite control. They also observed hypogonadism, but there were some intriguing differences from the Turkish patients. Their three siblings had profound central hypothyroidism and also needed to be treated for dwarfism in childhood with growth hormone replacement. This means that the lack of the leptin receptor in humans causes two abnormalities, neither of which have been seen so far in human leptin-deficient individuals. There is therefore a lack of parallelism between not having leptin secreted and not having appropriate leptin receptors. There may therefore be some constitutive activity of the leptin receptor that operates in the absence of leptin. It is intriguing to note that Porte's neuroendocrinologic analysis of the brains of *db/db* mice seemed to involve more marked abnormalities of POMC activities than those observed when leptin alone is missing as in the *ob/ob* mice. An alternative explanation is that some other ligand is stimulating and modifying the activity of the leptin receptor under normal circumstances.

It is valuable to compare the display of endocrinologic features in humans with those observed in mice. Leptin deficiency as such induces very strong parallels in terms of fat mass, appetite control, and gonadal function in the two species. However, the murine body temperature is lower as is the energy expenditure in the *ob/ob* mice, whereas there is much less evidence for this in humans. The other distinction is in relation to length. The *ob/ob* mice are short whereas all the homozygous leptin-deficient patients we studied were between the 70th and 90th percentile for height. There is, therefore, absolutely no evidence of impaired growth in the human homozygotes. Furthermore the *ob/ob* mice have a markedly activated hypothalamic pituitary adrenal (HPA) axis, whereas in the human leptin deficiency this axis is modestly, if at all activated. This implies that there are differences in the neurophysiologic wiring of neuroendocrinologic pathways in mouse and man. Knowing the effect of corticosteroids on growth, insulin resistance, and glucose metabolism, it seems reasonable to consider the selective activation of the HPA axis as in mouse but not in man as a possible explanation for the growth retardation in mice; this may also reveal why the metabolic responses of mice seem to be more marked than those observed in the human condition.

We now have experience of treating four children with leptin. We have found a remarkable reversal of massive obesity as soon as we begin graded leptin injections with dosage being gradually increased from very small amounts on the basis of individual responses.[8] The children tend to develop antibodies to the leptin but we are still obtaining very effective responses with an exclusive loss of fat mass and, as they grow and mature,

an increase in lean tissue. The effects on energy expenditure are intriguing. Measurements of sleeping metabolic rate measured in a calorimetric chamber show a drop over the first year of treatment with leptin, but accurate measurements of total energy expenditure, using D_2O^{18} reveal that the total energy output is maintained. This suggests that there has been an increase in physical activity in response to leptin therapy and this is just what the parents described: the children become more physically active as a spontaneous response to leptin therapy and weight loss. More intriguing is the response in appetite. We have now developed a system for testing the children's hunger. We provide children with a massive 4000 kcal feast for breakfast and they are allowed to eat as much as they like as we monitor their intake. It is evident that a 2-year-old leptin-deficient child can eat the equivalent of a small adult female's energy requirements for a day in a single breakfast. They only seem to stop eating when the stomach is so physically distended that they cannot eat any more! This massive ingestion at a single meal is evident on repeat testing, but within a week of leptin therapy there is an 85% reduction in food intake—a very dramatic demonstration of the effect of a single molecule on a complex human behavior. As therapy continues, we have been able to transform an immobile massive 9-year-old girl into a relatively normal, if still somewhat overweight, 12 year old.

From the leptin treatment trials it is not as yet clear whether we can obtain a graded response to increasing doses of leptin. It is valuable to assess this because leptin may be an evolutionary signaling system concerned only with the transition between the fasted and the extremely starved state when leptin levels fall markedly. At higher leptin plasma levels there may be no further response and this plateau in responsiveness at modest leptin levels may mean that the use of leptin for treating ordinary obesity is not a useful therapeutic approach. Rather than attempting to assess the impact of graded inputs of leptin, we decided on an alternative strategy that involved seeing whether we could find patients with constitutionally lower leptin levels and considering what influence these levels had on their fat mass. We therefore assessed 14 heterozygous carriers from our first three families. We also visited Birmingham, their hometown in the United Kingdom, and found people there from the same area of the Punjab with the same migratory history and socioeconomic circumstances. These people served as a carefully matched control group. We discovered that the 14 heterozygotes had a higher BMI than expected with a highly significant increase in the percentage of those classified as obese. They also had a much lower serum leptin level so that in these heterozygotes their leptin concentration per unit BMI was approximately one-fifth of that seen in the control individuals. If one considers the relatives without any gene abnormalities, they conform in terms of their serum leptin levels with the normal values, whereas the heterozygotes are markedly different. Curiously, those with very low leptin levels tend to be the older individuals, i.e., over 60 years of age. We then applied the Deurenberg equations, validated in different populations around the world, to predict the body fat mass of these individuals on the basis of their age, sex, height, weight, and ethnic origin. When we directly measured body composition in normal individuals from the Punjab they conformed to the Deurenberg equations, but we found that fat mass in heterozygotes was underestimated by 30%. These heterozygotes with one copy of the abnormal leptin gene have low serum leptin levels per unit of fat mass and not only have increases in BMI, but also have a greater increase in body fat mass than that predicted from the higher BMI values.[9] This suggests that individuals with a moderate reduction in leptin levels could have biologic consequences, which implies that there may be a proportion of people in society who have lower plasma leptin levels than expected for their BMI; this could be significant even if they do not have a currently recognized leptin mutation.

When we considered the reproductive status of our homozygous leptin-deficient children, we found that the older girl, at 9.5 years, had prepubertal levels of gonadotrophins. She also had a bone age of 12 years, so she should already, in endocrinologic terms, be entering puberty. After one year of leptin treatment, she gradually increased her basal and stimulated gonadotrophins with clear nocturnal pulses of leutinizing hormone (LH) and follicular stimulating hormone and during the next 6 months she developed robust mid-pubertal levels of LH with appropriate breast, pubic hair, and axillary hair development. She also started menstruating. In response to leptin the progression of normal puberty has now occurred.

It has been suggested that increments in leptin might be the signal for the onset of puberty, so we were concerned, when we gave leptin to a younger child, that we might precipitate aberrant changes in the reproductive axis. We have, however, been reassured by the fact that after a year of treatment, there is absolutely no sign of any activity of the hypothalamic pituitary gonadal axis in any of the younger children. Leptin appears to be acting as a metabolic gate that allows children to go into puberty but does not specifically precipitate puberty at an aberrant age. Increments in leptin at an appropriate age may indeed be an important trigger.

If we now consider the pathways of leptin signaling in the brain and those that involve the POMC system,

human studies have again given us a new understanding of the human control of body weight. Genetic changes in this signaling system were first described by Krude et al.[10] who found two children with bright red hair, very pale skin, and adrenal failure at birth with no production of cortisol; later both had severe hyperphagia and obesity. They had essentially no function related to their POMC gene. They lacked ACTH and therefore had adrenal failure. They had no skin pigmentation because of a lack of α-melanocyte stimulating hormone (α-MSH) and they had no POMC-derived peptides in their brains. We have recently detected a heterozygous POMC mutation Arg3267Gly that appears to be more than twice as common among obese as opposed to lean subjects (personal observations, Cambridge, 2001). This mutation disrupts a dibasic cleavage site in POMC and leads to the production of a fusion protein comprising β-MSH and β-Endorphin. Interestingly, this fusion peptide seems capable of acting as a partial antagonist at the MC4 receptor (see below). Thus, a relatively common missense variant in POMC may contribute more generally to the genetic susceptibility to obesity.

Mutations in the MC4 receptor are found in approximately 5% of children with severe early onset obesity.[11,12] These mutations are dysfunctional when tested in vitro and the children with these abnormalities are huge. They not only have a major increase in fat mass, but also in their lean body mass with an acceleration of linear growth. Thus a 9-year-old boy with this a homozygous mutation in the MC4 receptor is the same height as his 16-year-old brother. When we developed a simple index of hyperphagia by assessing the spontaneous amount of food ingested at breakfast and divided the amount by the child's lean body mass, it was clear that the four children with a homozygous mutation were hyperphagic but not as massively hyperphagic as children with complete leptin deficiency. This infers that there are leptin pathways independent of the MC4 receptor system that control food intake in humans. When we looked at the heterozygotes in the families with the MC4 mutations, we found that they also were overweight or obese and had hyperphagia. In the older heterozygotes, however, the hyperphagia assessed both objectively and subjectively, seemed to be moderate and adults with this MC4 mutation described themselves as being very hungry as children but not having abnormal hunger as adults. On this basis, there seems to be some developmental relationship with the function of the MC4 receptor in terms of food intake.

We also observed that the children with homozygous MC4 mutations were very hyperinsulinemic, i.e., analogous with the observations seen in mice with the MC4 receptor knockout.[13] When we matched individuals with heterozygous MC4 mutations for their degree of obesity, we found that at that degree of obesity the fasting insulin levels were approximately twice that expected in normal individuals. We also found that bone densities in the homozygous children were well above the 95th percentile for their age and size, again with a tendency for their bone densities to drift down toward to the expected median levels as they get older. These studies on the MC4 receptor, as well as the leptin system, reveal the complex interrelationships between the leptin signaling pathway and a number of physiologic controls in terms of pituitary function and insulin homeostasis. Clearly the new findings, e.g., in relation to the MC4 receptor, open new opportunities to look at molecular control points—of great interest to the pharmaceutical industry—but they also reveal subtle and interesting differences between humans and our experimental animals. This amplifies the value of painstaking clinical research that, over the next few years, should reveal new insights into the control of eating behavior and of the proportions of bone, other lean tissues, and fat mass before and after puberty.

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