A historical perspective on leptin

Douglas L Coleman

As the only child of a self-employed and largely self-taught Canadian radio-and-refrigeration repairman, I spent much of my childhood investigating how things worked by taking them apart. As I grew older, I developed a keen interest in science, and, while I was an undergraduate at McMaster University, my scientific interests converged on biology, geology and chemistry. During this time, a very dynamic biochemistry professor encouraged me to attend graduate school at the University of Wisconsin and study biochemistry. Upon receiving my PhD from the University of Wisconsin in 1958, I knew that the prospects of returning to employment in Canada were poor, and I was offered a position at the Jackson Laboratory in Bar Harbor, Maine. When I accepted this position, my plan was to remain at the Jackson Laboratory for one or two years to further my education in biology, especially genetics and immunology. As things turned out, however, the Laboratory provided a very fertile environment—with excellent colleagues and world-class mouse models of disease—and I spent my entire career in Bar Harbor. I never dreamed that I would work on obesity and diabetes or that my research would one day be deemed important enough to be considered for a major scientific award.

In 1958, only one obese mutant mouse—the *ob/ob* mouse—was known[1]. It had been discovered in 1950 and characterized as a model for mild diabetes, but it was not the subject of any ongoing studies by other investigators at the Jackson Laboratory. The *ob* mutation is located on chromosome 6, and the mouse is characterized by massive obesity, marked hyperphagia and mild transient diabetes. In 1965, a new obesity mutant—the *db/db* mouse—was discovered. I was asked to assist in characterizing this new mutant mouse and, in particular, comparing it to the *ob/ob* mutant. We found that the *db* mutation is on chromosome 4 and, like the *ob/ob* mutant mouse, the *db/db* mouse develops marked obesity and hyperphagia, but, unlike the *ob/ob* mutant, it develops severe, life-shortening diabetes[2]. **Figure 1** illustrates the striking obesity of the *db/db* mutant mouse compared with a normal littermate at eight weeks of age. The *db/db* mouse is much larger than the normal mouse (30 grams compared with 20 grams), and the extra weight is all adipose tissue. Because both *ob/ob* and *db/db* mice become morbidly obese with similar speed, the *db/db* mutant shown in this figure looks just like an *ob/ob* mouse; to the unaided eye, the two mouse types are phenotypically identical[2].

When I began to characterize this new mutation, I wondered whether some circulating factor might control the obese phenotype. For example, could a factor produced in a normal mouse prevent or remediate the metabolic abnormalities? Conversely, might a factor produced by the *db/db* mutant mouse cause obesity in a normal mouse? If this hypothetical factor were carried through the blood, I reasoned, I could test for its presence by linking the blood supplies of the various mouse strains. Fortunately, two of my colleagues at the Jackson Laboratory were using parabiosis to characterize anemia mutant mice and were pleased to instruct me in this technique. Parabiosis requires the surgical joining of two mice by skin-to-skin anastomosis from the shoulder to the pelvic girdle. Wound healing and cross circulation is established in two to three days. To avoid a vigorous immune-mediated rejection, this technique requires that the mice be on the same genetic background, an issue that would delay some desired pairings (see below). Most parabiosis experiments fail, either because there is no factor or because the factor exchanged is insufficient, owing to the lack of major blood vessels in the skin to mediate the cross-circulation.

Because the *ob/ob* and *db/db* mutant mice were on different genetic backgrounds, my first parabiosis experiment involved the joining of

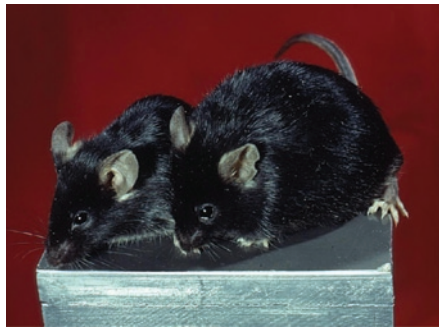

Figure 1 An obese *db/db* mouse (right) next to a normal mouse (left).

the *db/db* mutant to a normal mouse[3] (**Fig. 2a**). After what seemed to be good wound healing and a healthy-looking parabiotic pair, I was surprised to see that the normal mouse died. My immediate thought was that I was a pretty poor surgeon, but repeated attempts consistently yielded the same outcome: only the normal mouse in the pair died. Encouraged by this stereotypic pattern, I initiated a more detailed characterization of the normal partners and found that, after about one week, their blood sugar concentrations had declined to starvation levels. Moreover, at necropsy, the normal mice not only consistently lacked food in their stomachs and food remnants in their intestines but also had no detectable glycogen in their livers. In marked contrast, the diabetes partners consistently retained elevated blood sugar concentrations, and their stomachs and intestines were distended with food and food residues. This was my 'Eureka' moment! These results led me to conclude that the *db/db* mouse produced a blood-borne satiety factor so powerful that it could induce the normal partner to starve to death, even in the face of the limited cross-circulation between parabiotic pairs. Despite my excitement, my colleagues and most of the scientific community remained largely unconvinced.

At about the same time as these first parabiosis studies, a new obese mutant mouse spontaneously emerged[4]. This mouse did not

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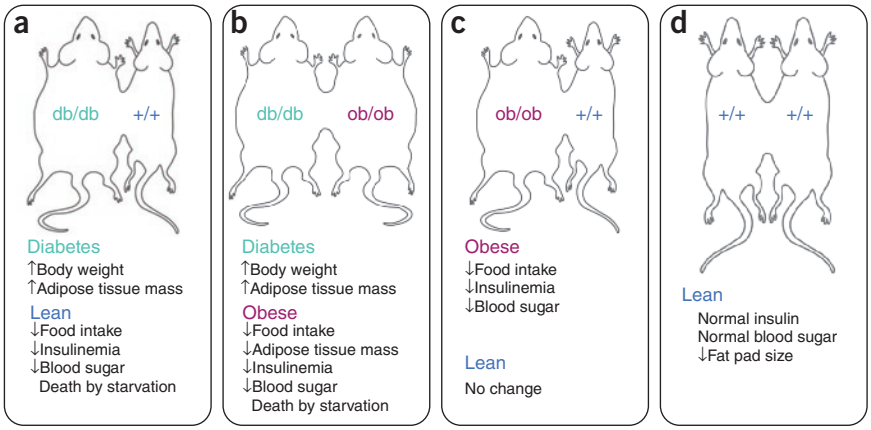

Figure 2 Summary of the parabiosis experiments. (**a**–**d**) Four different parabiotic combinations and the main phenotypes observed in each mouse in the pair.

have diabetes and therefore appeared identical to the *ob/ob* mutant, but, like the *db/db* mutant, the mutation mapped to chromosome 4. This new mutation arose in an unrelated strain, but when crossed into either the C57BL/Ks inbred background (in which the *db/db* mutation was maintained) or the related C57BL/6 inbred background (in which the *ob/ob* mutation was maintained) it caused markedly different disease syndromes—leading me to wonder whether the inbred background could mediate the severity of the disease. To answer this question, I placed the diabetes mutation on the C57BL/6 background and the obese mutation on the C57BL/Ks background by five cycles of cross-intercross breeding, thereby producing two new congenic lines[5]. I found that both the *ob/ob* and the *db/db* mutations, when maintained on the C57BL/Ks inbred background, produced identical syndromes: marked obesity with severe life-shortening diabetes. In contrast, when maintained on the C57BL/6 background, both mutations produced obesity but with only mild and transient diabetes. These studies clearly established that the severity of the diabetes depended on modifying genes in the inbred background. Interestingly, the modifying genes remain unknown to this day, although a great deal of effort has gone into identifying them.

Knowing that two genes on separate chromosomes produce identical syndromes strongly suggested that these genes mediate a common metabolic pathway. Having both types of mutant mice on both inbred backgrounds permitted the parabiosis of the *ob/ob* mutants with the *db/db* mutants without fear of rejection (**Fig. 2b**). This experiment would allow me to answer a question that had always nagged at me: was the normal parabiont starving because it was continually being dragged around by the larger *db/db* partner and never had a chance to eat? Parabiosis of mutant mice of the same weight would address this issue. These additional parabiosis experiments revealed that the *ob/ob* and *db/db* mutant mice responded similarly to the procedure regardless of inbred background. After collateral circulation developed, blood sugar concentration in the *ob/ob* mutant mouse declined, eventually reaching starvation levels. Survival time ranged from 20 to 30 days. At necropsy, it was clear that the adipose tissue mass in the *ob/ob* parabiont had decreased and that neither food nor food residue could be found in the stomach or intestinal tract of the *ob/ob* partner. By contrast, the *db/db* mutant was gorged with food and gaining weight. Consistent with the lack of food in the *ob/ob* partner, the food consumption of the pair was decreased to about that typical of a single *db/db* mutant mouse. These striking results clearly indicated that the *ob/ob* mutant mouse, like the normal mouse, recognized and responded to the factor provided by the *db/db* partner[6].

Several additional parabiosis and lesion experiments tied up loose ends. Parabiosis of *ob/ob* mutant mice with normal mice (**Fig. 2c**) not only slowed the weight gain in the *ob/ob* mutant mouse and decreased food consumption of the pair to that seen in normal-with-normal parabionts but also resulted in a pairs of parabionts that survived for months, until the end of the experiment. This study showed that the factor produced by the normal mouse was probably the same as that produced by the *db/db* mutant, but in insufficient amounts to be lethal. My overall conclusions from the parabiosis studies were that the *db/db* mutant mouse overproduced a satiety factor but could not respond to it—perhaps owing to a defective receptor—whereas the *ob/ob* mutant recognized and responded to the factor but could not produce it.

Further studies with *db/db* mutant mice that were lesioned in the ventromedial nucleus or the arcuate nucleus of the hypothalamus (or both) suggested that the receptor for this satiety factor is found in these brain areas[7]. Additional support for the idea that a satiety factor acts on a receptor in the brain came from a study in which rats with lesions in the ventromedial nucleus of the hypothalamus became hyperphagic and obese[8]. When lesioned rats were parabiosed with normal rats, the normal rat lost weight and failed to grow but did not die.

Despite these clear results, many of my colleagues and many in the obesity field maintained the dogma that obesity is entirely behavioral, not physiological. On the basis of these experiments, however, some investigators did accept a physiological basis as an underlying cause that contributes to obesity, and the hunt for the satiety factor became a race. None of the early satiety factor candidates (cholecystokinin, somatostatin, pancreatic polypeptide and others) stood up to rigorous experimentation, and I continued my own attempts to identify the factor. In control parabiosis studies using two normal mice (**Fig. 2d**), I had found that the pairs remained active and healthy for four months (until they were culled). But, at necropsy, they did show one abnormality: the fat pads were smaller in size than those isolated from unparabiosed normal mice. This suggested to me that the factor might be a component of adipose tissue. Although the factor was produced by adipose tissue, my time-consuming attempts to isolate it proved futile, as I concentrated my efforts on individual fatty acids or lipid extracts of adipose tissue.

Following a tour-de-force positional cloning exercise carried out over many years, the long-sought satiety factor was definitively identified by Jeffrey Friedman[9]. This satiety factor was named leptin and, with the subsequent cloning of the leptin receptor, the field exploded. Essentially all of the predictions made from the parabiosis experiments were verified: *ob/ob* encodes a blood-borne hormone (leptin) that functions in a negative feedback loop to control adipose tissue mass by modulating appetite; the *db/db* gene encodes the leptin receptor; leptin is produced in adipose tissue; and the leptin receptor is expressed primarily in the hypothalamus. These discoveries not only changed the prevailing theory about obesity, from being caused by a lack of willpower to being caused by an imbalance of hormone signaling, but also showed that adipose tissue is not just a useless and unwanted fat storage site but rather an important and essential endocrine organ.

In humans, many leptin deficiency syndromes are responsive to leptin replacement therapy. As leptin is involved in numerous

pathways, the effects of too little leptin are widespread (including infertility, impaired immune function, decreased insulin response and altered homeostasis). Although leptin mutations are not common in humans, leptin therapy in the few people who lack an active form of this hormone is a godsend, transforming them from morbidly obese individuals living in a state of perceived starvation into much leaner individuals with a more normal lifestyle. Leptin therapy has also successfully treated patients with reduced adipose tissue mass resulting from, for example, lipodystrophy or amenorrhea. However, leptin therapy is not a panacea for curing obesity, because most obese humans, who have normal genes for leptin and its receptor, are leptin resistant. Despite extensive efforts in many laboratories, it remains unclear why ordinary obese people become resistant to their own leptin. As we learn more about the molecular mechanisms that govern this crucial hormone, it is likely that combination therapies involving leptin will prove efficacious in other diseases. Indeed, exciting preclinical work from Roger Unger's laboratory raises the possibility that leptin supplementation therapy might be beneficial in the treatment of type 1 diabetes[10].

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COMPETING FINANCIAL INTERESTS

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