

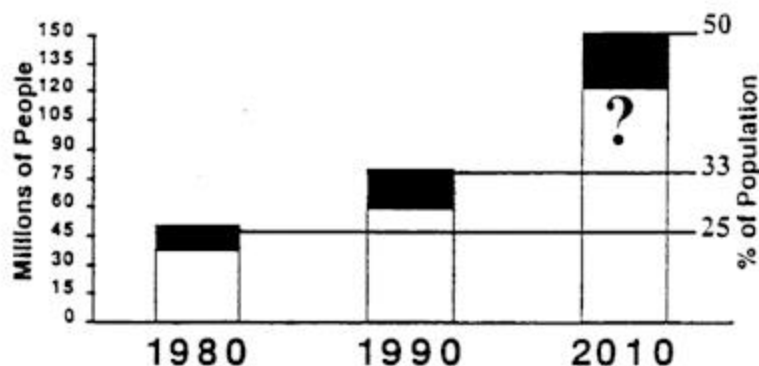
WEIGHT REGULATION AND THE ROLE OF LEPTIN

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A portion of this outline has been taken, with permission, from a Medical Grand Rounds lecture on the genetics of obesity given by Rody Cox, M.D. at U.T. Southwestern, March 7, 1996.

Obesity has become a world-wide epidemic, particularly in developed countries. It is associated with a number of serious morbidities, including Type 2 diabetes, osteoarthritis and hypertension. The current cost of obesity/Type 2 diabetes syndromes to the U.S. economy is staggering - estimated by some to be - \$ 1 00 billion annually. As alarming as these facts are, it is projected that if the present trend continues, by the year 2,010 some 50% of the U.S. population will be obese, and that of these - 30 million will be afflicted with Type 2 diabetes (Fig. 1).

FIG. 1:
Obesity □
and NIDDM ■
in the U.S.



The fundamental problem in obesity is simply stated:

ENERGY IN > ENERGY OUT

Much debated, however, has been the reason for this imbalance. To be sure, the obese individual is consuming more calories than are needed for essential activities. However, it is equally clear that not all individuals are "made equal" in terms of how they dispose of ingested calories, such that for the same dietary intake some will be more predisposed than others to gain weight. Herein lies the importance of genetic factors. Thus, in studies of dizygotic twins reared together or apart the correlation for body mass index ($BMI = \text{weight in Kg} / \text{height (m)}^2$) was found to be - 0.25 in each case. However, for monozygotic twins this value rose to -0.75 regardless of whether they

were reared together or apart. The fact that the value did not reach 1.0 suggests that genes and environment contribute ~75% and 25%, respectively, to the control of BMI.

Another strategy for determining how genetic factors play a role in fat accumulation is to study differences between people in response to a well-defined positive or negative energy balance. The amount of weight gained or lost can be determined, as well as the physiological and biological correlates of the response to changes in energy balance. To assess the possibility that a person's genotype is involved in his/her response to long term overfeeding, twelve pairs of young adult male monozygotic twins were studied (Bouchard *et al.*, *N. Eng. J. Med.* **302**: 1477-1482, 1990). All of the subjects were sequestered and observed during the hundred day period of this investigation (Table 1). The initial phase of the study was a two-week period during

Table 1. Protocol for Long-Term Overfeeding of Monozygotic Twins

- A. Twelve pairs of young adult male identical twins were sequestered
- B. Base-line daily caloric intake at a stable body weight was established during a 14-day observation period.
- C. During the next 100 days each subject consumed 1,000 kcal more than his baseline intake for 6 days a week
- D. The total excess amount each subject consumed was 84,000 kcal

which the subjects ate freely in a special dining room. All foods selected were recorded, weighed and evaluated for their caloric content by a dietitian. Each subject's habitual daily energy intake under conditions of a stable body weight and body composition was determined from the fourteen-day record of food intake. This value was considered to be the baseline for the study. After the two-week baseline period all subjects were overfed 1,000 kilocalories per day for six days a week for a total of 84 days during the hundred-day period. Therefore, the total excess that each subject consumed was 84,000 kilocalories. Table 2 summarizes the results of overfeeding. The average gain in body weight was 8.1 ± 2.4 kilograms; however, the range varied from 4.3 to 13.3 kilograms. Within each pair of twins the response to overfeeding was similar with respect to body weight, percentage of fat mass and estimated subcutaneous fat.

Table 2. Effect of 100 Days of Overfeeding in 12 pairs of Male Monozygotic Twins

| Averages* | |
|--------------------------------------|------------------------|
| Gain in body weight | 8.1 ± 2.4 kg (SD) |
| Range | 4.3 to 13.3 kg |
| Ratio of fat mass to fat free mass | 0.17 increased to 0.22 |
| Estimated change in subcutaneous fat | 76 increased to 129 mm |

*Statistical significance $P < 0.001$ for all above values

Table 2 (cont)

| | |
|------------------------------|-----------------------|
| Gain in fat mass | 5.4 kg or 52,220 kcal |
| Gain in fat free mass | 2.7 kg or 2,754 kcal |
| Calculated energy dissipated | 29,000 kcal |

However, between pairs of twins the variance was approximately threefold. Table 2 also shows the average gain in fat mass of 5.4 kilograms, which can be accounted for by 52,220 kilocalories of over feeding. The gain in fat-free body mass was 2.7 kilograms, which can be attributed to 2,754 kilocalories and a calculated average energy dissipated of 29,000 kilocalories. These figures are averages because the subject who gained the most weight (13.3 kilograms) had minimal evidence of energy dissipation, whereas the subject who gained the least weight (4.3 kilograms) had only 40% of the extra calories deposited as fat or body tissues. The subjects who gained more fat than lean tissue tended to gain more weight and to deposit more fat in visceral and truncal-abdominal areas. The implication of truncal-abdominal obesity and excessive abdominal visceral fat for insulin action, plasma lipid and lipoprotein levels, and their relations to mortality and morbidity are of considerable clinical interest. The most likely explanation for the resemblance between identical twins in their response to overfeeding is that a person's genotype is an important determinant of adaptation to prolonged energy surplus. All subjects consumed the same micro-nutrients and had the same positive energy balance of 84,000 kilocalories. All subjects also kept to the same relatively sedentary schedule during the period of overfeeding. Therefore, differences in the efficiency of weight gain probably resulted from individual variations in the preferential storage of energy as fat or as lean tissue as described in Table 2.

Complementary studies to the overfeeding experiments have been carried out on monozygotic twins who were fed a base level diet and were exercised to induce a negative energy balance. The twins were exercised on a cycle ergometer twice a day for 50 minutes per session while consuming a basal diet for a period of 22 days (Poehlman *et al*, *Eur.J Appl. Physiol.* **56**:398-402, 1987) or 100 days (Bouchard *et al*, *FASEB. J*, **6**: a1647 (Abstr.), 1992). The exercise prescription was designed to induce an extra energy expenditure of 1000 kilocalories while maintaining energy intake at baseline throughout the study. The results confirmed the overfeeding studies, with significant intrapair resemblance in the loss of body weight and fat mass as well as regional fat distribution phenotypes. The interpair differences in monozygotic twins showed marked variances, just as the overfeeding studies had. Taken together, these results support the thesis that there are individual differences in the tendency toward obesity and in the distribution of body fat. The similarities within a pair of monozygotic twins and the variance between different sets of twins strongly suggest a major genetic component.

Studies with animal models.

It was suspected a half century ago that the central nervous system (CNS), in particular the hypothalamus, plays a pivotal role in the regulation of body weight. In 1953, Kennedy went further, postulating that body weight is regulated through a lipostatic mechanism involving an interaction between

the hypothalamus and some factor in equilibrium with stored fat (*Proc. Royal Soc. London (Biol.)* **140**: 578-592, 1953). Subsequently, Hervey surgically linked normal rats, allowing partial exchange of blood between them (parabiosis), and electrically lesioned the hypothalamus of one member of each pair (*J. Physiol.*, **145**: 336-352, 1959). The lesioned animals became hyperphagic and obese, while their partners developed anorexia and died of starvation. Hervey reasoned that, with increasing adiposity, a humoral factor accumulates and serves to control food intake through a feedback interaction with the hypothalamus; such a factor would, by definition, be ineffective in the lesioned rats, but it would be passed via the circulation into the non-lesioned parabionts and would suppress their food intake.

With the advent of genetically obese mice (*ob/ob* and *db/db* strains; see Fig. 2), it became possible to test the effect of parabiosis between the fat and lean animals in various combinations. From a classic experiment of this type (Fig. 3), Coleman inferred that the *ob/ob* mouse must lack the circulating satiety factor, while the *db/db* strain fails to respond to it, thus accounting for the similarity of the two phenotypes when present on the same genetic background (*Diabetologia*, **9**:294-298, 1973).

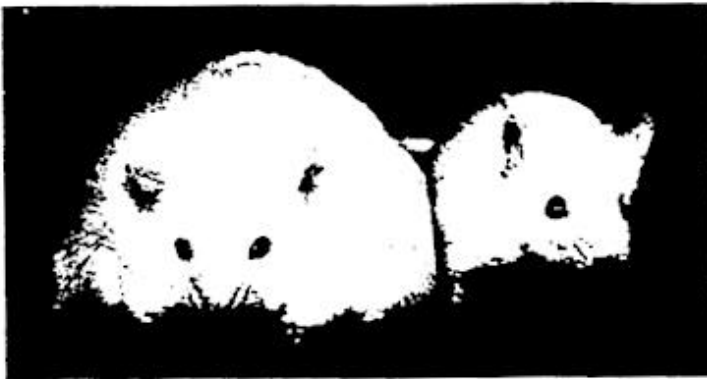


FIG. 2: A *db/db* mouse alongside its lean littermate.

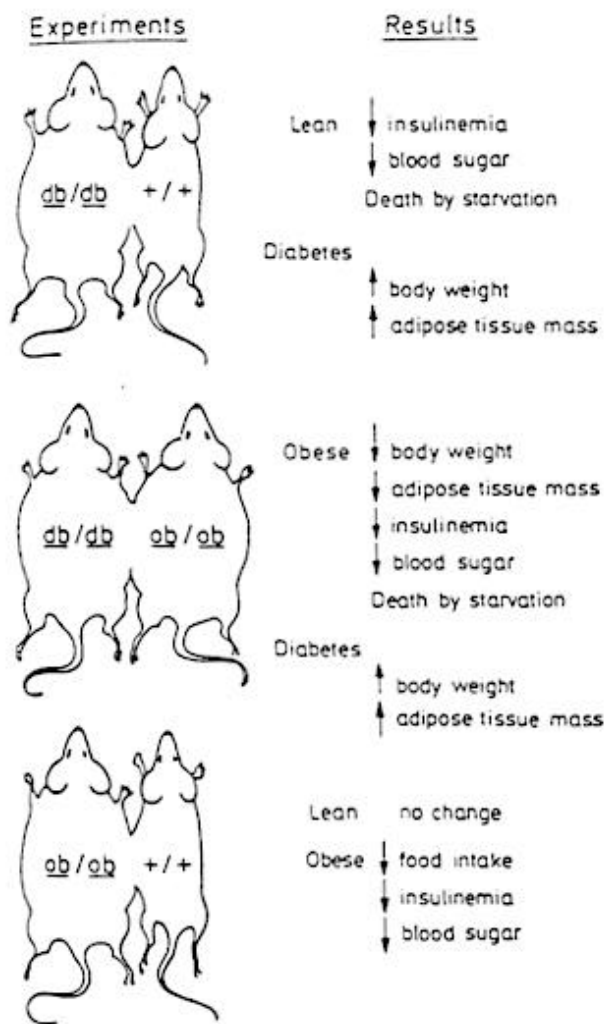


FIG. 3: Classic parabiosis experiment of Coleman in 1973.

Twenty years later there occurred a major breakthrough that has had an explosive impact on the field of obesity research. This wave of excitement was triggered by the momentous announcement from Friedman and colleagues that they had identified the mutation in the *ob/ob* mouse (*Nature*, **372**: 425-432, 1994). It turned out that the normal *ob* gene is expressed only in fat tissue; it encodes a protein named leptin (from the Greek word leptos, meaning thin) of molecular weight - 18kDa and containing a 2kDa cleavable leader sequence, indicating that it is a secreted product (this has since been amply confirmed). Leptin is highly conserved among vertebrates, the mouse and human homologues being 84% identical at the amino acid level. The mutation in the C57BL/6J strain *ob/ob* mouse introduces a stop codon into the leptin mRNA, causing failure to produce the normal protein and, interestingly, a 20-fold overexpression of the useless mRNA in white fat.

Soon thereafter, the leptin receptor was characterized both in the mouse and human. It exists in a variety of forms arising from differential splicing of the receptor transcript. The so-called short forms (lacking the long intracellular domain) are expressed in multiple tissues, whereas the long form (containing the extended intracellular domain believed to be necessary for signaling) is expressed primarily in the hypothalamus (Fig. 4).

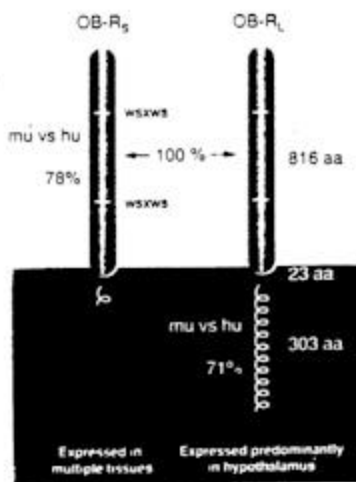


FIG. 4: The short (OB-R_s) and long (OB-R_L) forms of the leptin receptor are shown schematically.

WSXWS, Trp-Ser-X-Trp-Ser motif-, mu vs hu, murine versus human; aa, amino acids. From Tartaglia *J Biol Chem.*, **272**, 6093-6096, 1997.

Importantly, a mutation in the leptin receptor gene produces a truncated version of the hypothalamic receptor in the *db/db* mouse, resulting in its inability to produce the appropriate signals in response to leptin (Tartaglia *et al*, *Cell*, **83**:1263-1271, 1995; Lee *et al*, *Nature*, **379**: 632-635, 1996). A mutation in the leptin receptor gene has also been found in the Zucker fatty (*fa/fa*) rat, another animal model of obesity.

Consistent with the postulated role of leptin in the control of body weight, administration of the recombinant material to *ob/ob* mice (where leptin is absent) led to a prompt reduction of food intake, increased energy expenditure and loss of body fat (Fig. 5). As expected, the same maneuver was totally ineffective in *db/db* mice or Zucker *fa/fa* rats, both of which lack a functional leptin receptor.

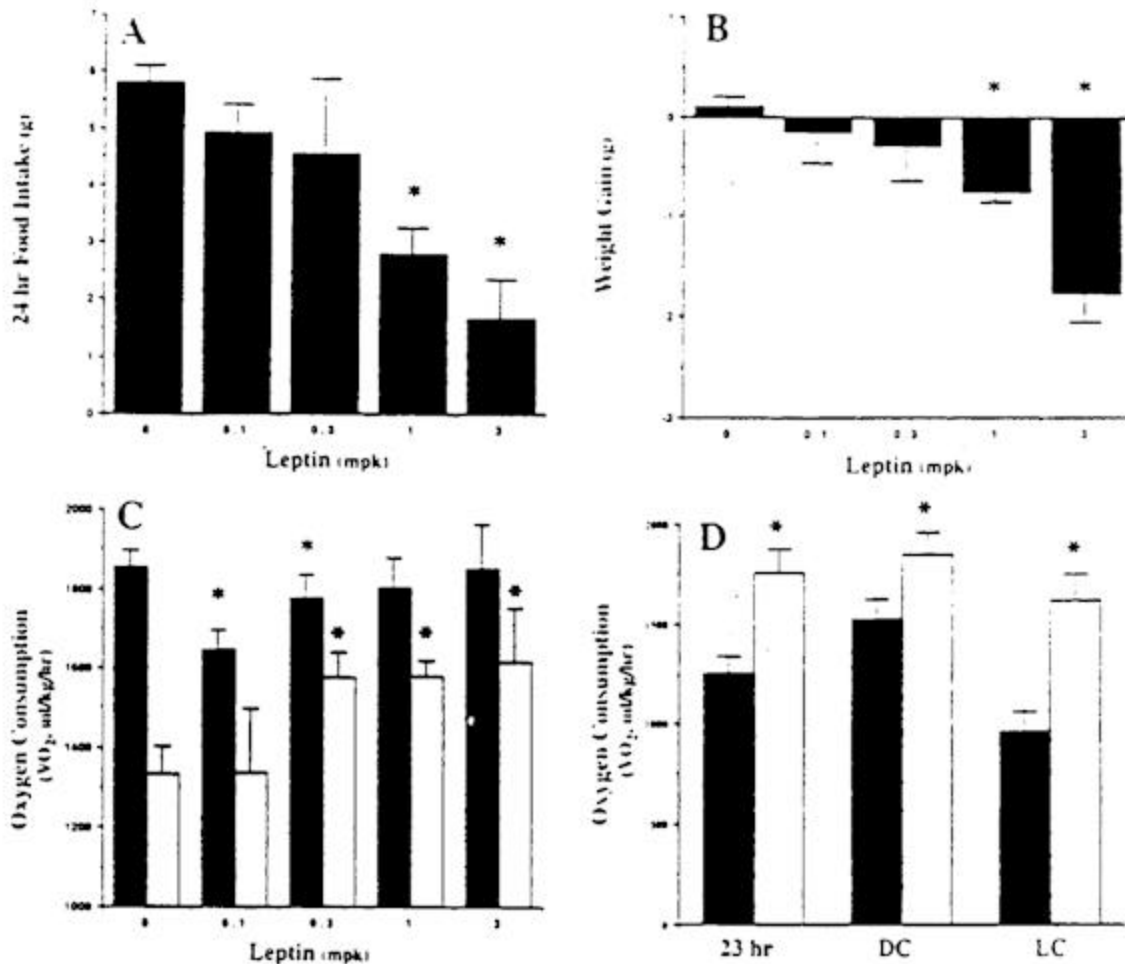


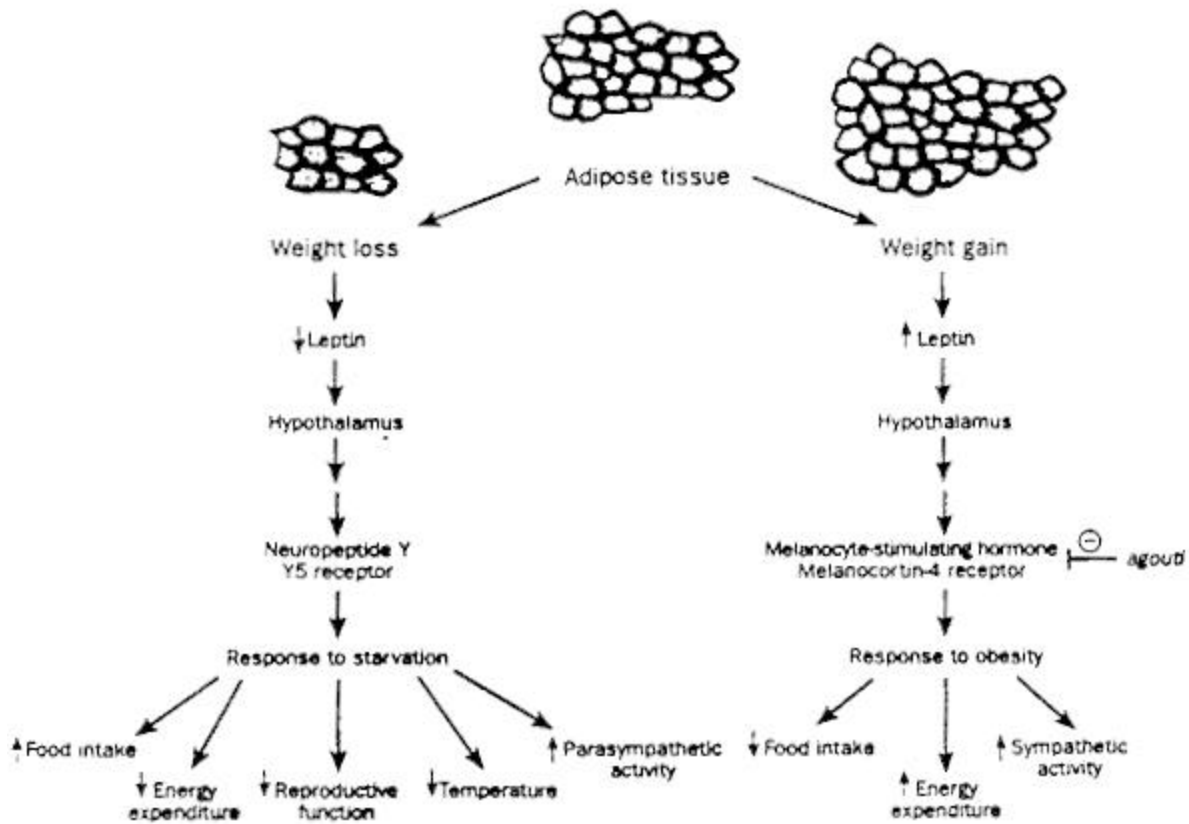
Fig-5: Dose-dependent effects of leptin (0.3-3 mg/kg ip) in *ob/ob* mice. A: effect on food intake. B: effect on body weight gain. C: effect on O₂ consumption in dark cycle (filled bars) vs. light cycle (open bars). D: comparison of O₂ consumption in leptin (3 mg/kg ip; open bars)-treated vs. pair-fed (filled bars) *ob/ob* mice. Values are means \pm SE, $n = 4$ in each group. mpk, mg/kg; DC, dark cycle; LC, light cycle. * Values significantly different from saline-treated group ($P < 0.05$).

From Hwa *et al* - *Am. J Physiol* **272**, R1204-R1209,1997.

How does leptin work?

Available evidence suggests that, in an overall sense, leptin is secreted from fat cells as adipose tissue mass expands and, by interacting with its receptor in the hypothalamus, triggers neurally mediated downstream events (possibly involving altered dynamics of neuropeptide Y and melanocortin receptor systems) to (a) increase sympathetic outflow from the CNS; (b) increase energy expenditure; and (c) to reduce food intake. Conversely, with starvation all of these changes are reversed (Figs. 6 & 7).

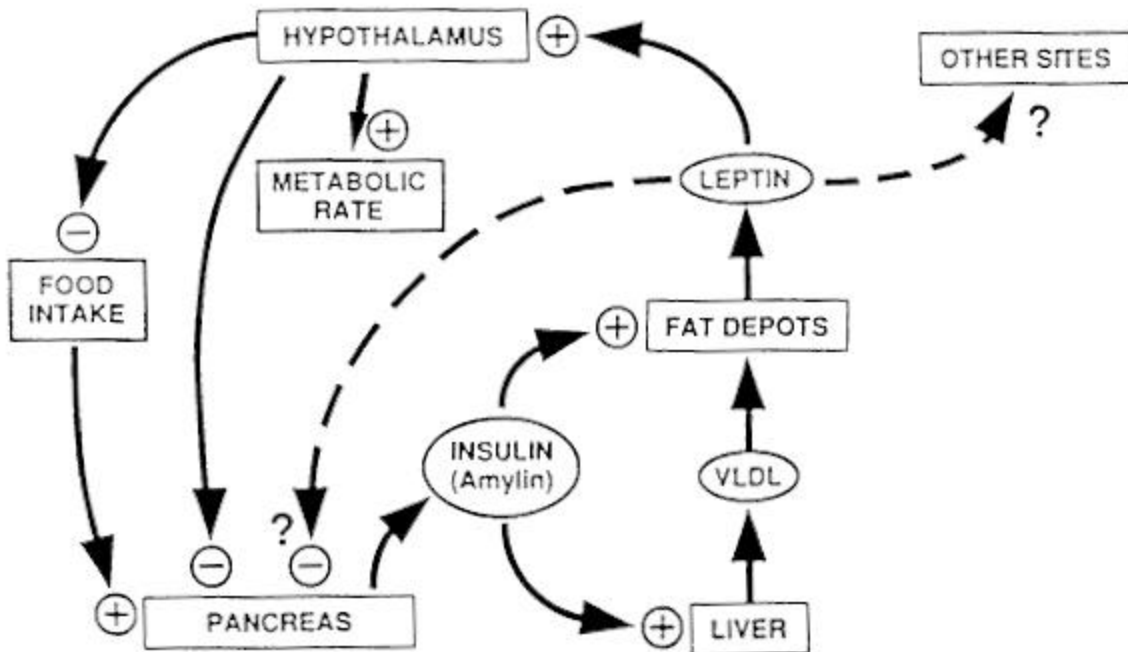
Fig. 6



Leptin allows the body to maintain constant stores of fat. A loss of body fat (starvation) leads to a decrease in leptin, which in turn **leads** to a state of positive energy-balance whereby food intake exceeds energy expenditure, and other responses are also seen. Conversely an increase in adiposity leads to an increase in the levels of leptin and a state of negative energy balance, whereby energy expenditure exceeds food intake. Fan *et al.* and Huszar *et al.* have shown that the melanocortin-4 (MC-4) receptor and its ligand, melanocyte-stimulating hormone, are necessary for the biological response to obesity. And Erickson *et al.* have bred mice with mutations of neuropeptide Y (NPY) to *ob* mice, and they show that NPY is an important component of the biological response to low levels of leptin and starvation.

From Friedman - *Nature*, **385**, 119-120, 1997.

Fig. 7: Potential interactions in lipostasis



What about human obesity?

Genetic predisposition to obesity in humans is almost certainly heterogeneous in origin. Predictably, the exciting advances made in the last 3 years from studies with animal models of obesity (see above) set off an intensive search for mutations in the genes for leptin and/or its receptor among obese humans. To date, only one such mutation has been found. This was shown to involve a frame shift in the leptin gene of two related children (cousins from a highly consanguineous family) that results in the production of a truncated, inactive leptin molecule (Montague *et al*, *Nature*, 387:903-908, 1997). Both subjects suffered from severe, intractable obesity at an early age. It is likely that both will also respond to exogenous normal leptin with correction of their hyperphagia and obesity. For the most part, however, it appears that the problem in the vast majority of obese humans lies not at the level of leptin or its receptor, but at some other step/s in the leptin circuitry, i.e., they are leptin resistant. Clear evidence to this effect is seen in the striking positive relationship between body fat content and circulating leptin levels in lean and obese subjects (Fig. 8).

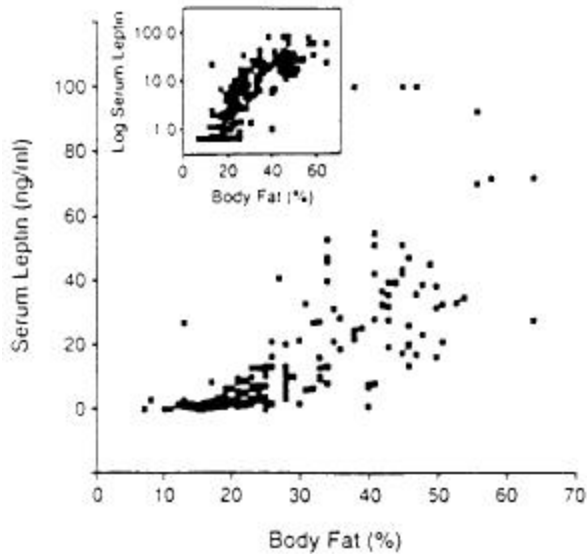


Fig. 8: Relationship between serum leptin level and body fat content in humans.

From Campfield *et al* - *Horm. Metab. Res.* **28**, 619-632, 1996.

A number of possibilities to account for obesity in humans are currently under active investigation (Table 3).

Table 3. Possible derangements in human obesity.

1. Defect in leptin's accessibility to its hypothalamic receptor.
2. Failure of leptin to suppress hypothalamic neuropeptide Y (NPY) synthesis.
3. Abnormalities in the melanocyte stimulating hormone (MSH) - melanocortin 4 (MC-4) receptor system in the hypothalamus.
4. Mutations in the β_3 -adrenergic receptor on fat cells and possibly other tissues.
5. Defects at the level of uncoupling proteins (UCP1, 2, 3 ...).

Regardless of what the genetic factors responsible for human obesity ultimately turn out to be, the question arises as to why the condition is becoming so prevalent. An attractive theoretical explanation is the "thrifty gene" hypothesis which argues that in more primitive times a genetic predisposition to lay down excess fat had survival value under conditions of food deprivation (droughts, hurricanes, tornadoes etc.). However, with increasing affluence of society, the almost unlimited availability of calorie-rich food, and the replacement of exercise by the automobile, the "thrifty gene" becomes a liability. According to this view, the fundamental problem underlying the current epidemic of obesity/Type 2 diabetes syndromes is societal and one that will predictably be difficult to correct even with modern medicine. Nevertheless, it is evident that the discovery of leptin has electrified the field of obesity research. Understanding precisely how this hormone works represents an exciting challenge for the future and could conceivably pave the way for novel pharmacological approaches to the treatment of a seemingly intractable medical problem.