GENETICS OF OBESITY

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INTRODUCTION

As Shakespeare wrote "does destiny shape our ends"-and perhaps our middles?

Obesity is an epidemic desease in the United States and other developed countries. Studies show that a 20% increase in body weight is associated with severe morbidities including non-insulin dependent diabetes (NIDDM), hypertension, osteoarthritis and other diseases (1-3). There are six to ten million people with NIDDM in the USA and approximately half the males and 70% of the females suffer from obesity. Weight loss is associated with amelioration of the diabetes and hypertension. With respect to etiology, the debate has centered on nature versus nurture. To what degree is obesity genetic versus acquired due to due to environmental factors, given the frequency with which obesity occurs it is likely that the causes are heterogenous and both heredity and environment play roles. Today I would like to review the evidence that obesity and the distribution of fat in the body has a significant genetic component. Obviously a quantitative trait as body weight must have a variety of genetic determinants and there is no simple Mendelian inheritance associated with the amount of fat accumulated and its distribution. Moreover, environmental factors also play a role. We will try to partition the influence of genetics and environment on obesity as determined by a variety of human and animal studies.

As general internists' we tend to regard an obese patient as responsible for his adiposity and there is some truth in this notion. However, the "field may not be level" as genes appear to play an important, if not a major role in predisposing an individuals body weight. I will just review selected family and twin studies that pertain to this issue. Recent work in the molecular biology of obesity has provided explanations for certain genetic forms of obesity that occur in mice. The relevance of these findings to human obesity and the potential role that these factors may play in determining body weight in humans will be discussed.

OBESITY PHENOTYPES

During the past fifteen years it has been recognized that obesity is not a homogeneous phenotype since fat accumulation occurs in different distributions. Table 1. outlines four types of obesity. Type 1 is characterized by excess total body fat without any particular

Table 1. Obesity Phenotypes

Type I	**Excess body mass or percentage of fat**
Type II	**Excess subcutaneous trucal-abdominal fat (android)**
Type III	**Excess abdominal visceral fat**
Type IV	**Excess gluteofemoral fat (gynoid)**

concentration of fat in a given part or area of the body. The remaining three types are associated with excessive accumulation of fat in certain areas of the body, that is they are based on the anatomical distribution of body fat. Type 2. is an excess subcutaneous fat on the trunk particularly in the abdominal area and is equivalent to the so called male type or android fat deposit. Type 3. is characterized by an excessive amount of fat in the

abdominal visceral area (abdominal visceral obesity). The fourth type of fat distribution is gluteofemoral obesity and is that observed primarily in women and is sometimes referred to as gynoid obesity. Therefore excess body fat can be stored primarily in a truncal-abdominal or in the gluteal and femoral areas or in the abdominal area or visceral. Propensity to store fat in one particular area over another shows individual variation and as described in Bouchard and his associates study of over feeding monozygotic twins, there were clear differences in the areas in which excess fat was deposited (1). Several other studies confirmed the genetic influences in different anatomical distributions of excess body fat (2,3).

HERITABILITY AS DETERMINED BY FAMILY STUDIES

During the last sixty years or so a large number of studies have reported that obese parents have a higher risk of having obese children than do lean parents. However this does not constitute a clear demonstration that the obesity of the offspring is determined by genes inherited from the parents, since both generations share not only their genetic backgrounds but also their household milieu, as well as other environmental conditions. In order to address this issue there have been a number of studies of nuclear families as shown in Table 2 (4). The body mass index (BMI) is a measure of obesity, that has been used in many studies. It is the weight in kilograms divided by the height in square meters. Table 2 shows approximately seventy-five thousand pairs of individuals were used to compare

Table 2. Intrafamilial Correlations of Obesity

	Numbers of Pairs	Body Mass Index
Spouses	23,936	0.12
Father-offspring	19,632	0.19
Mother-offspring	23,954	0.20
Brothers	6,017	0.26
Sisters	3,858	0.26
Brother-Sister	9,278	0.21
Monozygote Twins	79	0.58

*Body Mass Index (BMI) = Weight in kg divided by height in M^2

Adapted from the Norwegian study (4)

familial correlations in spouses and in first degree relatives (4). As indicated in this table the correlations for BMI were 0.12 for spouses, 0.20 for parent offsprings, 0.26 for same sex siblings and 0.58 for monozygotic twins. Using a PATH analysis and based on the large number of relatives in this study it was computed that the BMI had a heritability of approximately 0.40. PATH analysis is a method used in genetic epidemiology to assess the relative contributions of genetic and environmental factors based on correlations computed among various pairs of relatives by descent or adoption. Several adoption studies in which BMI data were available from both the biologic as well as the adoptive relatives of the adoptee reported that the effects of a shared environment on BMI were relatively small and that genetic factors play a greater role in the familial resemblance of obesity (5-7). In a review of behavioral genetic studies relevant to obesity the authors also concluded that

experiences shared among family member appear largely irrelevant in determining individual differences in weight and obesity (8). These findings are somewhat at odds with a strong familial association of major affectors of excess body fat, that is energy intake and energy expenditure (9,10). Therefore the presumed negligible effects of environment should be interpreted with caution.

TWIN STUDIES

The adoption studies revealing that the body mass index or BMI of adoptee resembles that of their biological parents and siblings but not that of their adoptive parents supports the role of heredity in human obesity. The extent of the genetic contribution however is uncertain and as shown in Table 2. studies of monozygotic twin pairs shows a high degree of correlation in their BMI. Table 3. shows a correlation for body mass index (BMI) in

Table 3. Correlations for Body Mass Index (BMI)

Reference	Number of pairs	Spouses	Number of pairs	Diygotic Twins	Number of pairs	Monozygotic Twins
Norway (4)	23,936	0.12	90	0.20	79	0.58
QFS (11)	1,163	0.19	69	0.34	87	0.88
NIH (12)	ND	ND	113	0.32	121	0.74

Body Mass Index = weight kg/height M^2

Heritability = Intraclass correlations (12)

spouses, dizygotic twins, and monozygotic twins in three large studies (4,11,12). The Norwegian data is derived from the National Health Screening Service Family Study which was quite extensive (4). The second study was the Quebec Family Studies (QFS) which also involved large numbers of spouses and twins (11). The NIH study is from the National Heart Lung & Blood Institute twin study and is a multi-centered longitudinal study of 514 white male twin pairs examined during military induction at the mean age of twenty years and then again at ages forty-eight and sixty-three years (12). Of these 121 of 254 monozygotic pairs and 113 of 260 dizygotic pairs had complete data for all three examinations. In these three studies of twins reared together, the genetic contribution to the correlations of body mass index was estimated to be from 0.58 to 0.88. These values may overestimate the contribution of heredity because similarities in twins reared together may result from shared environment as well as shared genes. Therefore, the study of identical twins reared apart is generally considered to be one of the most effective design for distinguishing the importance of shared genes from that of a shared environment. Tables 4 and 5 show the results of two such studies. Table 4 shows the results of rearing monozygotic twins together or apart as in the case of twins separated by adoption (13). The first conclusion from these data on twins reared apart is that the amount of body fat is strongly influenced by genetic factors. The correlation between twins reared apart indicate that the correlation for body fat is approximately 0.60 and strongly suggests that these similarities in body fat may be accounted for by genetic components. This estimate of genetic heritability is similar to estimates from other twin adoption and family studies. The correlations between twins reared apart is less than 1.0 which indicates that environment also plays a significant role in the accumulation of body fat. In Table 5 both monozygotic and dizygotic twins were studied and the sexes were separated (14). The intra-pair correlations of the ninety-three pairs of monozygotic twins reared apart provides

Table 4. Correlations Between Rearing of Monozygote Twins (MZ)

Measures	Number of Pairs	Reared Together	Number of Pairs	Reared Apart
BMI	38	0.751	34	0.610
Height	38	0.951	34	0.872
Weight	38	0.819	34	0.634

Statistical significance, P=.0001 for all values

Ref. Price and Gottesman (13)

Table 5. Body Mass Index and Intrapair Correlations in Monozygotes and Dizygotic Pairs of Twins Reared Apart or Together

Type	Men			Women		
	Number of Pairs	Body Mass Index	Intrapair Correlation	Number of Pairs	Body Mass Index	Intrapair Correlation
Monozygotes						
Reared apart	49	24.8 ± 2.4	0.70	44	24.2 ± 3.4	0.66
Reared together	66	24.2 ± 2.9	0.74	88	23.7 ± 3.5	0.66
Dizygotic						
Reared apart	75	25.1 ± 3.0	0.15	143	24.9 ± 4.1	0.25
Reared together	89	24.6 ± 2.7	0.33	119	23.9 ± 3.5	0.27

± Values are means ± SD

Ref. Stunkard et al (11)

an estimate of genetic influences that were independent of environmental contributions. The correlations values for men were approximately 0.70 and for women 0.66 and strongly supports a major genetic influence on body mass index. These values are very similar to those obtained from twins who were reared together 0.74 and 0.69 for men and women respectively. The lean body mass index was similar among the monozygotic twins and among the dizygotic twins (14). This is reassuring as to the representativeness of the sample and its remarkable similarity to other studies involving families. The second finding of the studies of twins was the indication of significant non-additive genetic variance. The intra-pair correlations for monozygotic twins were more than twice those of dizygotic twins. One qualification to the study by Stunkard and his associates, is that it contained very few morbidly obese persons. Therefore, the relation of these results to obesity depends upon a middle range of values for body mass index rather than the extremes that characterize obesity. Several adoption studies have shown that the genetic influence extended across the range of weights from thin to very obese (5-7). It is important that the studies of twins reared together and reared apart should be interpreted in the light of the concept of heritability. Heritability does not imply an invariant phenotype, for example hair or eye color. However, it does described the genetic influences found among persons living in a particular range of environmental conditions. Under different environmental conditions the same genotype might produce different estimates of heritability. In other words, genes

do not influence quantitative traits such as obesity and intelligence in an absolute manner. The genotype determines a range of expression of each of these traits. And the environment can determine where in the range an individual's phenotype may fall or be achieved.

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 their response to long term over feeding twelve pairs of young adult male monozygotic twins were studied (1). All of the subjects were sequestered and observed during the hundred day period of this investigation (see Table 6). The initial phase of this study was a two week period during which the

Table 6. Protocol for Long-Term Overfeeding of Monozygotic Twins

A.	Twelve pair 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

subjects ate freely in a special dining room. All foods selected were recorded, weighed and evaluated for their caloric content by a dietician. Each subject habitual daily energy intake under the condition of a stable body weight and body composition was determined from the fourteen day record of food intake. This value was considered to the baseline for the study. After the two week baseline period all subjects were over fed 1,000 kilo calories per day for six days a week for a total of eighty-four days during the hundred day period. Therefore, the total excess that each subject consumed was 84,000 kilo calories. Table 7 summarizes the results of over feeding. The average gain in body weight was 8.1 $\pm$ 2.4 kilograms. However, the range of gain in body weights varied from 4.3 to 13.3 kilograms. Within each pair of twins the response to over-feeding was similar with respect to body weight, percentage of fat mass and estimated subcutaneous fat. However, between pairs of twins

Table 7. 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	0.13 increased to 0.22
Estimated change in subcutaneous fat	76 increased to 129 mm
*Statistical significance P < 0.001 for all above values	
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

From Bouchard et al. (12)

the variance was approximately threefold. The range for weight gain varied from 4.3 kilograms to 13.3. kilograms and is of particular significance. Table 7 also shows the average gain in fat mass of 5.4 kilograms which can be accounted for by 52,220 kilo calories of over feeding. The gain in fat-free body mass was 2.7 kilograms which can be attributed to 2,754 kilo calories and a calculated average energy dissipated of 29,000 kilo calories. 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 gain more fat in visceral and truncal abdominal areas. The implication of truncal-abdominal obesity and excessive abdominal visceral fat for insulin metabolism, plasmid lipid and lipoprotein levels and their relations to mortality and morbidity are of considerable and clinical interests (13). The most likely explanation for the resemblance between identical twins in their response to over feeding is that a person's genotype is an important determinate of adaptation to prolonged energy surplus. All subjects consumed the same micro-nutrients and the positive energy balance was maintained at 84,000 kilo calories for all subjects (1). All subjects also kept to the same relative sedentary schedule during the period of over feeding. 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 7. Variations in the components of energy expenditure of individual genotypes during relative inactivity are particularly interesting as they dissipate excess calories and mitigate weight gain.

Complementary studies to the over feeding experiments were carried out on monozygotic twins who were fed a baselevel 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 (15) or 100 days (16). The exercise prescription was designed to induce an extra energy expenditure of 1000 kilo calories 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 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 twin and the variance between different sets of twins strongly suggest a major genetic component.

METABOLIC RATE AND ENERGY EXPENDITURE

Reduced energy expenditure for a given energy intake level causes positive energy balance and this eventually leads to excess body weight and obesity. The factors that cause an individual difference in energy expenditure are important since studies have reported that many obese subjects do not seem to have a higher caloric intake than do their lean counterparts. Energy expenditure is a complex and multifactorial phenotype that comprises many components. These include the basal and resting metabolic rates, thermic effects of food, the energy expended during activities and the energy costs for a particular activity. There is some evidence that genetic factors may contribute to energy expenditure (17,18) and resting metabolic rate (19).

Resting metabolic rate is a major component of energy expenditure and may account for

approximately 60-70% of daily energy expenditure. Genetic studies regarding the heritability of resting metabolic rate are very limited. One study comparing monozygotic and dizygotic twins, revealed that concordance was always higher in the monozygotic twin than in the dizygotic (20). After adjusting for age, gender, body mass and body composition the heritability estimates derived from this study suggests that 40-70% of the variance in resting metabolic rate may be inherited. Other studies of monozygotic and dizygotic male twins found that differences in the resting metabolic rates were accounted for by differences in body weight (21). In this latter study, when metabolic rate was measured under condition of stress, the authors did find a significant genetic effect that was independent of body weight and accounted for approximately 20% of the variance in energy expenditures. Taken together the results of studies of heritability of resting metabolic rates suggests that there may be a significant genetic component, although further investigation is needed.

Beta, Adrenergic Receptor and Obesity

Recent studies have highlighted a highly complex and sophisticated system of regulating fat and energy balance. The adrenergic system plays a major part in controlling energy expenditures. Catecholamines mobilize energy rich lipids by stimulating lipolysis in fat cells and thermogenesis in brown adipose tissue and skeletal muscle. It has recently been discovered that a special adrenergic receptor Beta, is involved in this complex regulation. The Beta, is the principle receptor mediating catecholamine stimulated thermogenesis in brown adipose tissues. In humans these deposits are scattered about the great vessels in the thorax and abdomen. Brown adipose tissue differs from white adipose tissue in that it has large numbers of mitochondria containing a so-called uncoupling protein (UCP) which can stimulate oxidative phosphorylation and thereby increase the metabolic rate. The role of brown adipose tissue is to oxidize lipids and to produce heat and catabolize excess fat. White adipose tissue includes subcutaneous and visceral adipose tissue and is much more abundant than the brown adipose cells. White adipose tissue stores fat which can be mobilized by lipolysis to generate free fatty acids for use by other tissues. The Beta, adrenergic receptor is important in mediating the stimulation of lipolysis by catecholamines in the white fat cells of mammals including man. The Beta, adrenergic-receptor activity might be involved in predisposing for obesity by several mechanisms. The most direct would be a decreased thermogenesis in brown adipose tissue. To my knowledge there is no studies of thin and obese humans with respect to the amount of brown adipose tissue present or to the response of that tissue to catecholamines via the Beta, adrenergic-receptor. However, significance differences might well account for the tendency of obese subjects to have a reduced basal metabolic energy expenditure and thereby predispose them to increasing their fat stores. Furthermore, decreased function of the Beta, adrenergic-receptor in white adipose tissue could reduce lipolysis and cause retention of lipids in fat cell. This may be especially important in contributing to visceral obesity which is the form of regional fat accumulation that predisposes risks of cardiovascular disease and diabetes. Recent studies in Pima Indians have shown a mutation in the gene for the Beta, adrenergic-receptor that predisposes patients to obesity and non-insulin-dependant diabetes mellitus (NIDDM). The mutation is a missense mutation that results in the replacement of tryptophan by arginine at amino acid #64. This mutation resides in the first intracellular loop of the seven membrane spanning domains of the Beta, adrenergic-receptor (Fig 1). The receptor is coupled to guanine-nucleotide-binding (G proteins) and is localized in adipose tissue. Stimulation of the receptor by Beta adrenergic agonist activates adenylate cyclase which increases intracellular concentration of cyclic AMP and results in lipolysis

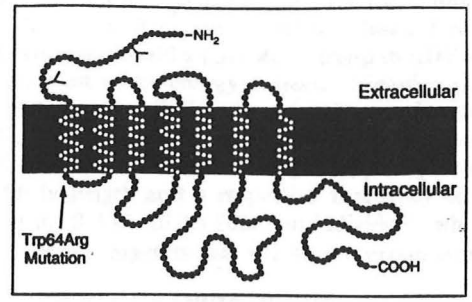


Figure 1. Diagram of the β_3-Adrenergic Receptor.
Each amino acid is shown as a circle. The Trp64Arg mutation appears at the beginning of the first intracellular loop.

and thermogenesis (25). The same mutation has been found in Finnish and French population where it was associated with the early onset of NIDDM and clinical features of insulin resistance as well as morbid obesity (26,27). Table 8 shows that Pima Indians who

Table 8. Characteristics of Pima Indians According to Genotype for B_3 Adrenergic Receptor

Character	Tryp 64 Arg Homozygote	Heterozygote	Normal Homozygote
No subjects	57	290	295
Diabetes %	72	60	60
Diabetes before age of 25 yrs %	9	3	3
Body Mass Index	35.2 ± 8.0	34.1 ± 7.9	33.9 ± 7.5
Difference from normal in adjusted basal metabolic rate	-82	-36	0

±SD

Adapted from Walston et al (25)

have Trp 64 Arg mutation develop diabetes at an earlier age, have somewhat higher BMI and most significantly have lower adjusted metabolic rates (25). Studies of the Beta$_3$ adrenergic-receptor and its potential role in obesity raise the possibility that selective agonists of this receptor might be useful in treating certain patients with obesity because they can enhance energy expenditure with few Beta$_1$ or Beta$_2$ adrenergic side effects. The findings of a mutation in the Beta$_3$ adrenergic-receptor are important since they support the role that a defect in brown and white adipose tissue might result in the development of obesity and its complications (25-27).

Mitochondrial Uncoupling Protein (UCP) and Obesity

Other targets for mutation that may contribute to the complex mechanisms of obesity in humans involves the mitochondria uncoupling protein (UCP) gene which uncouples oxidative phosphorylation in brown adipose tissue and helps generate heat (29). The UCP

protein is a 32 kilo dalton molecule that acts by uncoupling the usual link between fatty acid oxidation and the generation of ATP in mitochondria. Instead of harnessing the energy generated by fatty acid oxidation to add a phosphate group to ADP and create ATP, mitochondria with UCP dissipate most of the energy as heat. Ricquier and his associates have found that people who tend to gain weight have a natural occurring polymorphism in the gene that encodes UCP (32). They studied 261 subjects from 64 families of the Quebec Family Study population. A BcII restriction fragment-length polymorphism was identified with 2 alleles of 8.3 and 4.5 kb in length and a respective frequency of 0.28 and 0.72. There was a higher frequency of the 8.3 kb alleles found in individuals who tended to gain the most weight (Table 9). The results suggests that the 8.5 kb group may have a

Table 9. Correlation of UCP Polymorphism with Gain in Percent Body Fat over 12 Year Period

	With 8.3 allele n=27	Without 8.3 allele n=30
High gainers 11.2 ± 4.6%	16(62.1)	11(37.9)
Low gainers 2.6 ± 2.6%	9(32.1)	19(67.9)

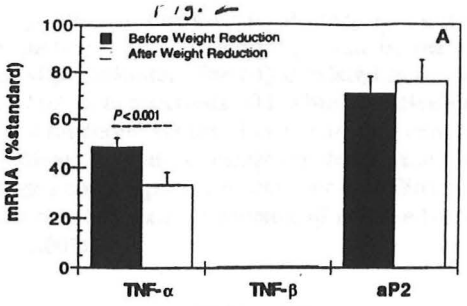
( ) are percentages

Adapted from Oppert et al (32)

predisposition to gain weight over time, although the numbers of patients studied was to small to form firm conclusions.

Tumor Necrosis Factor-alpha and Obesity

Recently tumor necrosis factor-alpha as been found to be over-expressed in obese individuals and this increase level of TNF alpha is associated with insulin resistance and abnormal glucose homeostasis (33). In this study the expression pattern of TNF alpha mRNA in adipose tissue from 18 controls and 19 obese premenopausal women was assessed by Northern blot analysis. The TNF alpha protein concentrations in plasma and in media from explanted adipose tissue were also measured by an immunoassay. The result showed that obese individuals expressed 2.5-fold more TNF alpha mRNA in fat tissue relative to lean controls. Moreover there is an increased and proportional secretion of TNF alpha-protein by cultured adipocytes from obese subjects. However, the TNF protein in serum was too low to be measured. The expression of TNF alpha mRNA correlated with the level of hyperinsulinemia which is an indirect measure of insulin resistance. Weight reduction resulted in improved insulin sensitivity and was associated with a concurrent decrease in TNF alpha messenger RNA expression in fat tissues (Fig 2). There have been no genetic studies on TNF expression in subjects who are obese. However, the results of the current study suggests a role for the abnormal regulation of this cytokine in the pathogenesis of obesity and related insulin resistance (33). The putative mechanism of TNF alpha effects is apparently mediated by a decreased ability of the insulin receptor to autophosphorylate after it binds insulin. This nearly abolishes the ability of the insulin receptor to phosphorylate insulin-receptor-substrate-1 the first link in the chain of signaling molecules that prompts muscle and fat cells to transport the GLUT-4 receptor to their surface. These alterations are a prerequisite for cells to take up glucose from blood. The reduced



expression of the gene encoding GLUT-4 and insulin sensitive glucose transport molecule present on the surfaces of fat and muscle cells lowers the ability of fat and muscle cells to respond to insulin by taking up glucose. This then leads to hyperglycemia and the development of non-insulin dependant diabetes (34).

Muscle Fiber Type and Obesity

Investigations of the genetic effects of energy expenditure associated with physical activity are also limited. An intriguing study of the correlation of energy expenditure with the slow muscle fiber-type I showed an inverse relationship to obesity (18). The hypothesis tested was that small imbalances between energy expenditure and energy intake may be the result of variations in the ability of muscle to metabolize fatty acids. This questions presupposes that there is a persistent difference between individuals in the catabolic biochemical pathways supplying energy. The body's single largest tissue mass is the skeletal muscles. Could it account for these differences in energy dissipation? It has been known for many years type I or slow muscle fibers are well endowed with mitochondria and work oxidatively with fatty acids as an important substrate. Type II fibers or the fast muscle fibers particularly those that are not adapted by regular exercise have fewer mitochondria and prefer the glycolytic pathway for energy supply. Proportions of these different muscle fiber types vary widely among individuals and certain large muscles such as the vastus lateralis of the quadriceps femoris may have as much as 96% or as little as 13% slow fibers. Since the respiratory quotient (RQ) is inversely related to the proportion of fat being combusted, if there are variations in muscle fiber type proportions that are important to individual variations in fatness, a difference in RQ might be detectable particularly during muscle work. This hypothesis was tested by biopsying 11 healthy men, age 21 to 50 years who were not taking part in competitive sports (18). Muscle biopsies were taken from the vastus lateralis through a needle. The stained muscle fibers were assessed as to ATPase activity and recorded as a percentage of slow fibers. Plainmetry with a graphics table was used to calculate the percentage area of the main muscle fiber class. Height, weight and skin fold thicknesses were also recorded as an estimate of the percentage of body fat. Figure 3 shows the relationship between the percentages of body fat and slow Type I fibers. The open circles in Figure 3 are data derived from Staron's study on muscle fiber type and obesity in untrained controls, weight lifters and endurance athletes (35). Plotting this data along with the authors' data shown in the closed circles clearly demonstrates an inverse correlation between the percentage of slow fibers and the tendency for obesity.

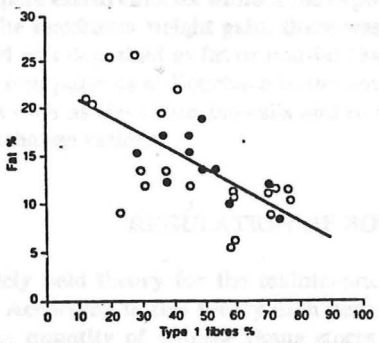


Fig 3—Relation between percentage body fat and percentage of slow (type 1) muscle fibres.

To further study this putative relationship

the authors propose to measure respiratory exchange ratios from which the metabolic respiratory quotient or RQ is derived. That is, the amount of CO_2 exhaled per unit of oxygen utilized per minute. The RQ is related to the proportion of fatty acid combusted. In this second set of experiments 50 healthy, relatively sedentary men exercised by a cycle ergometry at a moderate standard load while the respiratory exchange ratios (RERs) were measured. Mean oxygen consumption during the fixed load tests was about half the maximal oxygen consumption expected for a healthy man. Figure 4 shows that there is a direct relationship between the amount of adipose tissue and the RER (R = 0.538 with a p value of <0.001).

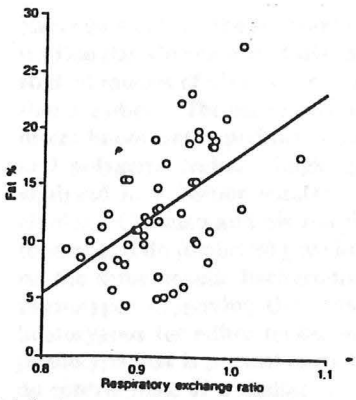


Fig 4–Relation between percentage body fat and respiratory exchange ratio.

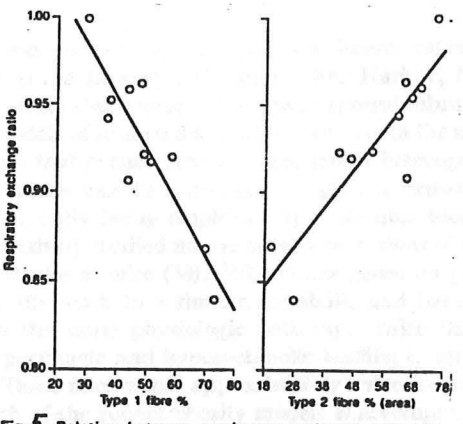


Fig 5–Relation between respiratory exchange ratio and percentage of slow fibres (left) or percentage fast fibre area (right).

In addition to the direct relationship between obesity and RER in the 11 men studied in the first of experiment, there was also a clear inverse relation between RER and the percentage of slow fibers (r = 0.894) and between RER and fiber area, this is shown in Figure 5. These correlations were strong even after exclusion of exercise variables such as heart rate, fitness and body size.

In the study of Bouchard, on the response to long term overfeeding in identical twins (1) it was clear that there was a major difference between monozygotic twin pairs and their ability to dissipate excess calories without the expected weight gain. In other pairs of twins, who showed the maximum weight gain, there was almost no dissipated calories and all of the excess food was deposited as fat or non-fat tissues. It would be of great interest in the well characterized patients of Bouchard to measure the proportion of slow fibers in major muscle groups such as the vastus lateralis and to carry out physiological measurements of respiratory exchange ratios.

REGULATION OF BODY WEIGHT

The most widely held theory for the maintenance of body weight involves the set point concept (36). According to this theory each person has an internal set point or adipostat that senses the quantity of adipose tissue stores and then regulate these stores. The internal set point modulates caloric consumption, physical activity, and thermogenesis. The

system that is central to regulating body weight and fat deposition. It requires a balance between energy intake and expenditure. This requires a system with afferent and efferent arms and a signaling whereby information coming from the adipose stores to a sensing mechanism, which is presumably in the hypothalamus, then adjust the homeostatic process to maintain adipose stores within the proper range. Thus the set point model implies the existence of 4 major components of energy homeostasis system: an afferent signal indicating the quantity and composition of energy stores, an efferent process regulating energy stores and expenditures, an efferent mechanism controlling feeding behavior and an integration system for these 3 components. Abnormalities in any of the components of this regulatory pathway could lead to obesity and inherited defects could provide the genetic contributions to obesity.

Early support for the set point concept was derived from seminal experiments carried out by Douglas Coleman and his associates at the Jackson Laboratory, Bar Harbor, Maine. Rodent models of obesity include 7 apparently single gene mutations in several inbred lines that are obese. The use of such animal models of human disease, particularly in the mouse, offers important experimental advantages that permit genetic dissection of heterogeneous and polygenic traits. Single gene mutations can be segregated in genetic crosses and analyzed in a fashion similar to that currently being employed by molecular biological cloning. Coleman and his associates intensively studied mouse obesity mutations of the ob (obese) and db (diabetes) gene in inbred strains of mice (38). When these genes are present on the same genetic background, ob & db result in a similar metabolic and behavioral phenotypes suggesting they function in the same physiologic pathway. Mice that are homozygous for either mutation are hyperphagic and hypometabolic leading to an obese phenotype that is present early in life. These mice weigh approximately twice as much as do control mice of a similar strain. Each of the rodent obesity models is accompanied by alterations in carbohydrate metabolism that closely resemble those of Type II diabetes in humans. The severity of the diabetes depends on the background of the mouse strain studied. Therefore the phenotype of the ob & db mice resemble human obesities in the way that diabetes is an intimate part of phenotype. The mutant mice eat more and expend less energy than do lean controls similar to human obese subjects. This phenotype resembles that seen in animals with lesions of the ventromedial hypothalamus, which suggests that both mutations may interfere with the ability to properly integrate or respond to the set point regulation described earlier. Fig 6. is a schematic representation of results of parabiosis experiments using normal, ob (obese) and db (diabetic) mice with cross-circulations. As shown in Fig 6. when normal mice have a cross circulation with db/db mice they become hypoglycemic with low insulin, they stop eating and die by starvation. This suggests that the db homozygous mouse is over-producing a substance that is the satiety factor and the normal mice respond by reducing food intake and energy expenditure. It appears that the diabetic partner produces but does not respond to the satiety factor that prevents overeating. These findings suggest that the db/db mice have an abnormal satiety center, but overproduce the satiety factor. When obese (ob/ob) mice were parabiotic with diabetic (db/db) mice the obese (ob/ob) partner lost weight and died of starvation while no abnormal changes were observed in the diabetic (db/db) partner. This suggests that the obese mouse is deficient in satiety factor but has a normal satiety center and can respond to the satiety factors produced by the diabetic mouse. Parabiosis of the obese ob/ob mouse with a normal mouse causes the obese mouse to reduce its food intake and diminishes the hyperinsulinemia and lower its blood sugar to near normal ranges. This suggests that the obese mouse is unable to produce a humoral satiety factor to regulate its own food consumption but that it has a normal satiety center since it can respond to the

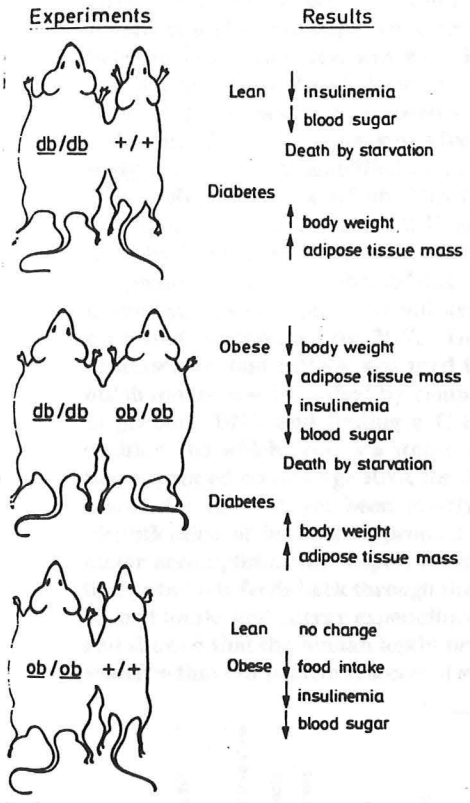


Fig. 6 Schematic representation of experimental results obtained between all combinations of normal, obese, and diabetes mice. Parabiotic pairs of like genotypes all survive and maintain their typical metabolic features

satiety factor produced by its lean parabiotic partner. The studies by Coleman and his associates identified the possibility of a humoral satiety factor and satiety center receptor presumably in the hypothalamus. These experiments although carried out in the 70's set the stage for the more recent efforts to identify the responsible molecules.

Cloning of the OB Gene

Coleman studies clearly indicated that the obese gene (ob) is located on chromosome #6 of the mouse and the diabetes gene (db) is located on chromosome #4. In order to isolate the ob gene by the technique of positional cloning, back-crosses and inter-crosses of mouse strains posessing the ob gene were carried out. Progeny segregating the ob/ob gene were established from more than a thousand informative meioses, providing a genetic resource for positional cloning of the ob gene. Mapping of molecular probes identified from several sources, that is from consensus mouse genetic maps, syntenic relationships between mouse and human genomes and from libraries prepared by chromosome micro-dissection localized molecular markers flanking the ob gene at a distance of 0.25 centimorgans (39-41). A major breakthrough occurred when Jeffrey M. Friedman and his group reported the positional cloning of the mouse obese gene (ob) and its human homologue (42). As shown in Fig 7 to

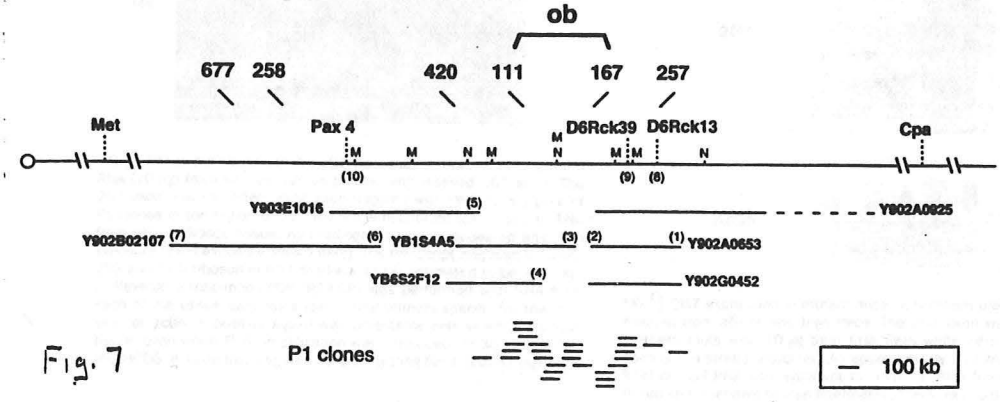


Fig. 7

isolate the mouse ob gene, DNA in the region of markers Pax-4 and D6Rck13 were used to construct a physical map. Yeast artificial chromosomes (YACs) corresponding to regions between Pax-4 and D6Rck13 were isolated and characterized. As shown in Fig 6 these YACs overlapped the ob locus with the exception of a small region between 2 and 3. Plasmid P1 clones were isolated carrying the missing genomic fragment. Using the technique of exon trapping, genes from this 650-kb sequence were isolated. A trapped exon designated 2G7 was amplified and hybridized to northern blots of various mouse tissues. This probe detected a 4.5-kb RNA found only in white adipose tissue as shown in Fig 8. The level of expression of the 2G7 exon was assayed in fat cells from two obese strains of mice by hybridization to northern blots as well as by RT-PCR. In one mouse strain designated SM/Ckc+^{Dac} ob^{2J}/ob^{2J} mRNA was absent as shown in Fig 9 when compared to the normal strains of mice. A second strain of obese mice designated as C57BL/6J ob/ob over-expressed the message for 2G7. The mutant mouse strain (C57BL/6J ob/ob) that over-expressed mutant mRNA was used to prepare cDNA by RT-PCR. The mutation in this ob/ob mouse was identified by comparing base sequence between normal and ob/ob mice in genomic DNA and finding a C to T substitution in the CGA codon for arginine at position 105 which creates a stop codon TGA. The mouse strain (SM/Ckc-+^{Dac} ob^{2J}/ob^{2J} that expressed no message RNA for the ob gene had a genomic rearrangement, that to my knowledge has not yet been clearly identified. The cloning of the ob gene and the identification of its protein product called leptin (from the Greek meaning "slim") is a major accomplishment. Leptin represents a secreted hormone produced by adipose tissue that putatively feeds back through the hypothalamus, where it mediates important controls of food intake and energy expenditures. Friedman's group also identified the human gene and showed that the human leptin protein is 84% identical to the mouse OB protein. The evidence that OB protein is secreted suggests that it is the circulating satiety factor that has

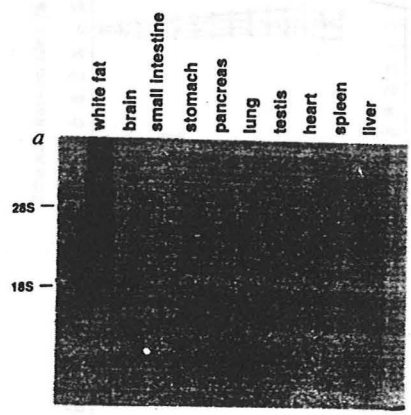


FIG. 8 Tissue distribution of the 2G7 transcript. *a*, Northern blot of total RNA (10 μg) from various tissues probed with labelled 2G7 exon. The 2G7 exon was identified using exon trapping with DNA from a pool of P1 clones in the region of *ob*. This probe hybridized specifically to RNA from white adipose tissue. Autoradiograph signals appeared after 1-h exposure (24-h exposure shown here). The transcript migrated between 28S and 18S ribosomal RNA markers and is estimated to be ~4.5 kb. *b*, Reverse transcription-PCR (RT-PCR) was performed with RNA from each of the tissue samples shown using primers specific for the 2G7 exon or actin. A positive signal was detectable only in white adipose tissue, even when PCR amplification was continued for 30 cycles.
METHODS. *a*, Exon trapping was done by ligating *Bgl*II/*Bam*H1 digestion

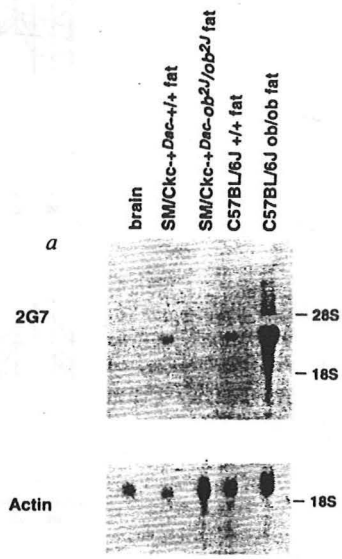


FIG. 9 2G7 expression in mutant mice. *a*, Northern blot of fat cell RN isolated from *obese* and lean mice. The 2G7 exon was hybridized t northern blots with 10 μg total RNA from white adipose tissue fror each of the strains indicated. An approximately 20-fold increase in th level of 2G7 RNA was apparent in white fat RNA from the C57BL/6 *ob/ob* strain relative to lean littermates. There was no detectable signa in RNA from the SM/Ckc-+^{Dac}ob^{2J}/ob^{2J} mice even after a 2-wee

been sought since the seminal experiments of Coleman (37,38). The identification of a nonsense mutation in an ob mouse strain that is deficient in the OB protein strongly supports the concept that the lack of leptin is the cause of obesity in the ob homozygous mouse. This is further supported by the same phenotype in a mouse strain that does not produce mRNA for leptin (42). The predicted sequence of the gene product has 167 aminoacids and the characteristics of a secreted protein.

Physiology of Leptin Action

Normal leptin was expressed in E. coli and purified to homogeneity as a 16 kilodalton monomer. Injection of the OB protein (leptin) into mice of different genotypes resulted in a dose and time dependent reduction in body weight for all groups of mice as shown in Fig 10 (43). Homozygous ob mutant mice lost 22% of their body weight when given 10mg/kg

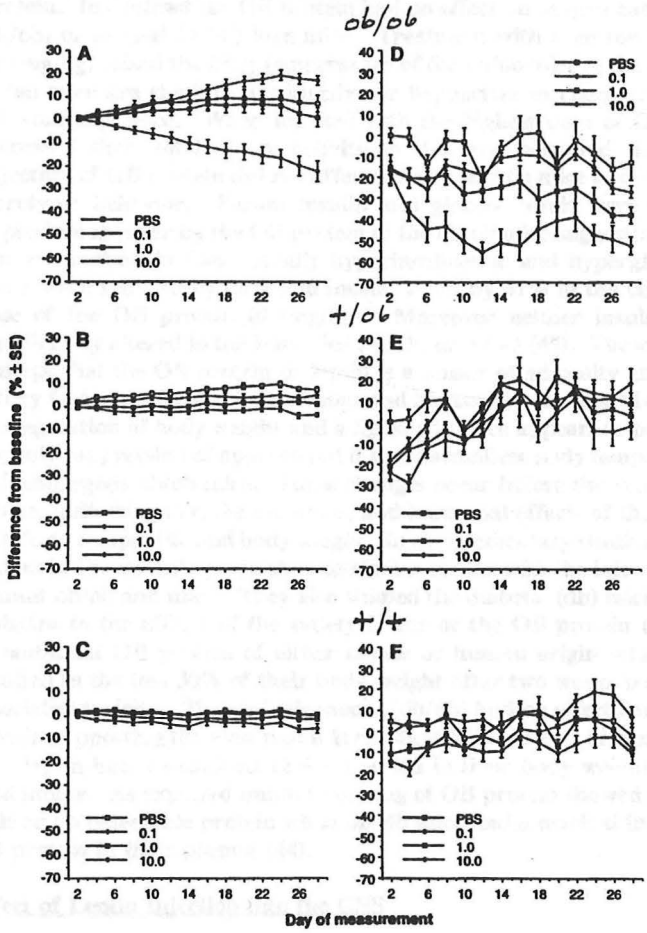


Fig. 10 Effects of OB protein administration on body weight in (A) C57BL/6J *ob/ob* mice, (B) C57BL/6J +/? mice, and (C) C57BL/6J +/+ mice. Numbers indicate doses (mg/kg per day). Body weight is expressed as the percent difference from the body weight on day 6 of baseline measurement. The first injection was given on day 1. Baseline weights for each group of PBS-treated animals were: *ob/ob*, 27.95 ± 1.34 g; +/?, 18.8 ± 0.23 g; and +/+, 21.7 ± 0.25 g. Shown in (D), (E), and (F) is food intake as a percentage of baseline consumption for the three groups of mice. The average baseline food intake values per day for PBS-treated mice were: *ob/ob*, 4.23 ± 0.16 g; +/?, 2.97 ± 0.075 g; and +/+, 4.0 ± 0.12 g. The baseline body weights and food intake of other dose groups were not statistically different from those of the PBS groups. Each test group contained 8 to 10 mice.

of OB protein. It should be noted that the rate of weight gain was also significantly altered by the OB protein in the heterozygous mice as well as in normal mice. The heterozygous mice receiving the highest does of OB protein (10mg/kg per day) showed a loss of 3.3% of their baseline body weight whereas the saline controls showed a 7.7% increase in their basal weight. In the homozygous normal mice the injection of the OB protein also decrease weight gain and there was no significant trend toward weight loss in the mice injected with a higher dose. These results suggests that the ob/ob mice are more sensitive to leptin than are the lean controls. The weight lost observed after injection of the OB protein was attributed in-part to reduction in food intake, moreover the OB protein also altered metabolic and endocrinological parameters in addition to suppression of appetite (43).

The ob/ob mice had significantly lower oxygen consumption than their lean counterparts and this parameter was normalized in those mice receiving the highest dose of the OB protein. In contrast the OB protein had no effect on oxygen consumption in heterozygotes (+/ob) or normal (+/+) lean mice. Treatment with even the lowest dose of OB protein (0.1mg/kg) raised the body temperature of the ob/ob mice to the level of lean mice. Mutant ob/ob mice are significantly inactive or hypoactive in comparison to the lean normal or heterozygous mice. When injected with the highest dose of OB protein the ob/ob mice increased their total motor activity to the level observed in their lean counterparts. Injection of OB protein did not affect activity in lean mice and did not induce any form of sterotypic behavior. Serum insulin and glucose levels were also decreased in a dose dependent manner by the OB protein in the ob/ob mice suggesting that pancreatic function was normalized in these usually hyperinsulinemic and hyperglycemic animals. Glucose levels were reduced by 66% and insulin levels by 41% in the ob/ob mice given the lowest dose of the OB protein (0.1mg/kg). Moreover neither insulin or glucose levels were significantly altered in the lean mice (+/ob, or +/+) (43). These data strongly support the concept that the OB protein or leptin is a sensor of adiposity and is the long sought after satiety factor predicated by Coleman and his associates. The pivotal role of OB protein in the regulation of body weight and adiposity in mice appears to be complex since it involves not only suppression of appetite but it also normalizes body temperature and serum glucose in homozygous ob/ob mice. These changes occur before the reduction in weight and food intake, indicating that the metabolic and hormonal effects of the OB protein may precede its effects on appetite and body weight. In complementary studies published simultaneously Halaas in Friedman's group showed similar results with the injection of the OB protein into mutant ob/ob and mice. They also studied the diabetic (db) mice, a mutant thought to be resistant to the effects of the satiety factor or the OB protein (leptin) (44). Injection of recombinant OB protein of either mouse or human origin into homozygous ob/ob mice resulted in the loss 30% of their body weight after two weeks treatment with no apparent associated toxicity. The diabetic mouse (db/db) had no effect from the injection of the OB protein supporting the view that it is resistant to the effects of leptin. Normal mice injected with leptin had a sustained 12% reduction in their body weight and exhibited decreased food intake. As expected immunoblotting of OB protein showed that the ob/ob mouse had little or no detectable protein while db/db mice had a marked increased in the level of the OB protein in their plasma (44).

<u>Effect of Leptin Injection into the CNS</u>

Campfield and his associates simultaneously published a study of recombinant mouse OB protein and the effects of injection into the central nervous system. A single dose of recombinant OB protein (1mcg per mouse) into the lateral ventricle through a

intracerebroventricular (ICV) cannula immediately stopped ob/ob mice from eating and the effects persisted during the remaining 6.5 hours of the experiment (45). ICV injections of OB protein into lean, normal or heterozygotes (+/ob) mice also resulted in a marked reduction in food intake. On the other hand ICV injection of OB protein did not reduce food intake or have an effect on body weight in the obese db/db mice. The demonstration that the mouse OB protein can alter feeding behavior and energy balance when placed directly in the lateral ventricle of the brains of ob/ob and lean (+/+) and (+/ob) mice suggests that one or more brain areas are among the target sites for the mouse OB protein. The inability of OB protein to alter feeding in the db/db mice supports the concept that the defect is in the satiety center. The identification of these brain areas will facilitate studies aimed at elucidating the neuronal pathway and networks and the underlying molecular mechanisms by which the OB protein can influence feeding behavior and energy balance (45).

Regulation of Leptin Synthesis in Adipose Tissue

Recent studies on regulation of the leptin gene in adipose tissue indicate that there is a diurnal variation, increasing during the night when rats or mice eat (46). The variation in leptin gene expression is linked to changes in food intake as fasting prevents the cyclic variation and is associated with a decrease in the leptin mRNA in adipocytes. Refeeding of fasted animals restores the leptin mRNA within four hours to levels observed in fed animals. A single insulin injection in fasted animals also increased leptin mRNA to levels of fed controls. Studies in primary adipocyte cultures and in rats show that insulin regulates leptin gene expression directly regardless of the glucose lowering effect in the media or the animals serum. It would appear from this study that insulin may play an important role in leptin gene expression following the ingestion of food (46). Other physiological and hormonal factors have also been showed to affect leptin gene expression. Glucocorticoids are known to have important metabolic effects and to modulate food intake and body weight. Pharmacological doses of glucocorticoids have a major catabolic action (47). The effect of large dose of glucocorticoid hormones on the expression of the leptin gene in adipose tissue indicated that leptin mRNA is rapidly induced by these hormones. The induction is followed by a concordant decrease in food consumption and body weight. These data suggests that the catabolic effect of corticosteroids on body weight and mass, and on food intake may be mediated in-part by alterations in leptin gene expression. Modulation of leptin expression may therefore constitute a mechanism through which hormonal, pharmacological and other factors help to control body weight homeostasis (47). The effect of cold temperatures on ob gene expression was studied in the epididymal fat pad of mice (48). Overnight (18hr) exposure of mice to a temperature of 4° C led to the disappearance ob/ob mRNA in this adipose tissue. Further studies showed that cold-induce loss of ob mRNA could occur in as little as 2-4 hours of exposure at 4°C. When mice were returned to a warm (24° C) environment there was a rapid stimulation in the expression of the ob gene with the return of normal levels of mRNA within 2.5 hours. It was found that the cold-induced suppression of ob gene expression was apparently mediated by the sympathetic nervous system. Studies with catecholamines including noradrenaline and isoprenaline produced results that were identical to those observed in the cold. The profound effect of cold on ob gene expression indicates that the ob system relates to energy expenditure as well as the satiety (48).

Leptin Levels in Normal Weight and Obese Humans

Leptin concentrations were measured by a radioimmunoassay in 136 normal weight subjects and 139 obese subjects whose body mass index was greater than 27.3 for men and 27.8 for women (49). The mean serum leptin concentrations in the 139 obese subjects was 31.3 ± 24.1ng/ml as compared with 7.5 ± 9.3ng/ml in the normal weight subjects (P>0.001). These results are shown graphically in Fig 11. Leptin mRNA was measured in abdominal subcutaneous adipocytes from 54 of the subjects. In the 27 obese subjects biopsied the leptin mRNA content was twice as high as in the 27 normal-weight subjects (29.0 ± 8.7 -vs- 1[illegible].8 ± 10.9 relative units, P=0.005) (49). Seven of the obese subjects were placed on an 800 kilocalorie diet daily and lost 10% of their initial weight in 8-12 weeks. The mean serum leptin concentrations decreased by 53% and the leptin mRNA content of adipocytes decreased by 38% after weight reduction. In these human subjects the correlation between body mass index or percent body fat and the plasma leptin level is highly significant for both the men and women. The mechanism by which the increase in body fat is translated into an increase in serum leptin appears to involve induction of the ob gene expression. Several other studies have found a significantly greater amount of leptin mRNA in adipocytes from obese subjects than in those from normal-weight individuals (49-52). There is heterogeneity in the leptin concentration among obese individuals. Some obese patients have extremely high leptin levels whereas others have levels similar to those observed in lean subjects. In obese patients who reduce their food intake, the smallest decrease in plasma leptin after weight loss, is seen in those whose starting levels were low. These findings suggests that in patients whose adipose tissues is secreting relatively less leptin into the circulation, adiposity has a quantitatively lesser effect on leptin levels. It is unclear whether patients with a high plasma leptin level are clinically different from patients whose plasma concentrations are low. There are no studies, as yet, on whether these groups would respond differently to recombinant leptin protein (50). It is possible that obese subjects with relatively low leptin levels might respond to exogenous leptin. The large fluctuations in serum leptin concentrations in the presence of relatively small changes in body weight suggests that leptin secretion may be regulated by other factors, in addition to the size of the adipose-tissue depot (49). When subjects are on a calorie restricted diet and in negative caloric balance reduced leptin may produce a signal to stimulate appetite. This would compound difficulties in adhering to a diet. Several potential signals described in the section on "Regulation of Leptin Synthesis and Adipose Tissue" (see above) may not be as strong a stimulus in humans as in rodents. Fasting serum insulin concentrations decrease during weight loss but the post-prandial rise in serum insulin during a period of feeding was not associated with significant change in serum leptin concentrations in humans. This result is different than that reported for rodents.

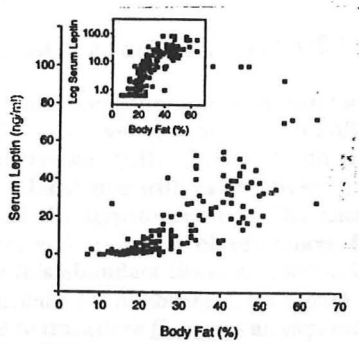


Figure 11 The Relation between the Percentage of Body Fat and the Serum Leptin Concentration in 136 Normal-Weight and 139 Obese Subjects.
The inset shows the natural log of the serum leptin concentration plotted against the percentage of body fat.

Although leptin may simply be a marker of the adipose tissue mass the data presented above are also consistent with the possibility that, in some cases, resistance, that is reduced sensitivity to its physiological effects, may lead to obesity and a compensatory increase in plasma leptin levels. Resistance could be partial or complete. If the resistance is partial high doses of the leptin protein may be required for bioactivity. The observed decrease in

leptin levels after weight loss may provide a partial explanation for the high recidivism rates among dieters. Thus reduced endogenous leptin levels after dieting may lead to a state of increased hunger and decreased energy expenditure. If this is true recombinant leptin might help to maintain weight loss after dieting(50). The data suggests that obesity in many humans may be more likely to be due to central mechanisms regulating food intake and energy expenditure rather than to defective signaling by adipocytes.

Identification of a Leptin Receptor, OB-R

Tartaglia and his collaborators searched for the putative leptin receptor by preparing highly purified ^{125}I leptin and a series of leptin-alkaline phosphatase fusion proteins to search for the leptin receptor (53). Coronal brain sections were prepared from mice and the tissue slices were incubated with radioactively labeled leptin which bound to the mouse choroid plexus and the hypothalamus. The choroid plexus is the site of cerebrospinal fluid production and transport of substances from the blood to the brain. Once they had identified this abundant tissue as a leptin binding sites, the investigators prepared a complementary DNA library from mouse choroid plexus. Plasmids carrying the cDNA were used to transduce E. coli as an expression system. Using as a probe the leptin-alkaline phosphatase fusion protein they identified an E. coli clone expressing a leptin-binding protein. Nucleotide sequencing of the entire 5.1 kb cDNA insert revealed a single long open reading frame which was predicted to encode a protein of 894 aminoacids. This polypeptide is a novel single membrane-spanning receptor that was called the OB-R. The predicted mature extracellular domain has 816 aminoacids. This extracellular domain of the OB-R protein has many features of the class 1 cytokine receptor family, and is most closely related to the gp 130 signal-transducing component of interleukin 6 (IL-6) receptor. The OB-R is also related to the granulocyte colony-stimulating factor (G-CSF) receptor and the leukemia inhibitory factor (LIF) receptor. Although the overall aminoacid sequence identity between OB-R and gp130 of IL-6 is only 24% the motif and conservation of other critical residues within this family of proteins was clearly evident(53). Using the radioactive cDNA as a probe, mRNA encoding this receptor is highly expressed not only in the choroid plexus but also in lung, kidneys and most significantly in the hypothalamus, the structure in which leptin site of action is believed to occur.

Identification of the gene encoding OB-R indicated that it mapped to murine chromosome 4 and is quite close to the diabetes db gene. The occurrence of the OB-R gene near the db gene is very exciting. The db/db mouse is obese and overproduces leptin suggesting that there is a receptor defect. Binding of leptin in db/db mouse brain sections is apparently normal (53). Recent studies have identified six alternatively spliced transcripts that encodes isoforms of the mouse OB-R with long intracellular domains (53a,53b,53c). The db/db mice also produce alternatively spliced transcripts, but one frequent isoform in the hypothalamus has a 106 nucleotide insertion that prematurely terminates the intracellular domain (53a,53b,53c). Morever a G to T transversion was identified in the genomic OB-R sequence in the db/db mice (53a,53b,53c). This mutation generates a donor splice site that converts an intronic 106 bp sequence into a novel exon retained in the OB-R transcript Fig 12. The authors predict that the long intracellular domain form of the OB-R is critical for initiating intracellular signal transduction. In the db/db mouse With truncated forms of the OB-R the failure or partial failure to initiate intracellular signal transduction leads to severe obesity observed in db/db mice (53a,53b,53c). It is also possible that OB-R serves a transport function, moving leptin from the peripheral circulation into the central nervous system. The choroid plexus is known to largely responsible for the generation of the

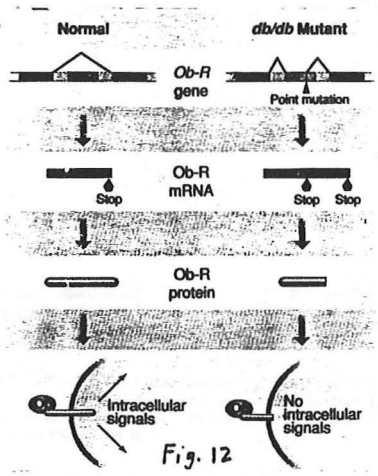


Fig. 12

cerebrospinal fluid and is one of the barrier's between the blood and the cerebrospinal fluid. The binding of leptin to choroid plexus membranes also may lead to the activation of afferent neural inputs that contribute to the networks that regulates feeding behavior and energy balance.

The demonstration that OB-R RNA is present in the hypothalamus as well as the choroid plexus and the mutation in db/db mice implicates this receptor directly in signal transduction within the hypothalamus. The observation that some obese humans and obese mice other than ob/ob have high levels of leptin and leptin mRNA relative to lean controls suggests that the majority of obesity is probably a consequence of leptin resistance, rather than inadequate amounts of leptin itself. Further studies of the OB-R may shed new light on the mechanism of leptin resistance in human obesity.

Role of the Hypothalamus in Obesity

The concept of a dual-center "hypothesis" for the regulation of food intake in an autonomic nervous system-mediated processes is probably too simplistic a theory. However it provides a convenient forum for discussing the regulation of appetite and body weight. The hypothesis proposes that the ventromedial hypothalamic nucleus (VMH) is a satiety center that inhibits the activity of the lateral hypothalamic area (LHA), a feeding center.

It is generally believe that the VMH is implicated in the regulation of the sympathetic nervous center, whereas the LHA mediates its effects through the parasympathetic nervous system. This theory is based upon studies involving bilateral destruction of each of these centers and then observing the phenotype of the animal. Bilateral destruction of the LHA is followed by reduction of food intake, the loss of body weight, and an increased activity of the sympathetic nervous system. On the other hand bilateral lesions of the VMH results in increased food intake, increased body weight and an exaggerated parasympathetic out-flow all resulting in the development of obesity. The accumulation of fat closely resembles the genetic mouse models of ob/ob and db/db mice. Obviously the division of satiety in feeding centers in a nuclei, the VMH and LHA respectively, is a simplification but one that is useful.

Fig 13 is a scheme for the "dual-center" hypothesis for the regulation of food intake and the autonomic nervous system. Destruction of the VMH produces hyperphagia in mice, humans and a variety of other animal species (54-57). This lesion is accompanied by enhanced vagal activity with increased secretion of insulin and glucagon from the pancreas. Metabolically, VMH lesions result in an initial state of insulin hypersensitivity and a marked increase in body weight, followed by a state of insulin resistance during the static phase when body weight is maintained. In addition to causing hyperphagia and obesity, lesions of the VMH disrupt the normal circadian rhythm of food intake and insulin secretion. During the metabolic changes, the sympathetic nervous appears to be inhibited. It is noteworthy that rats with VMH lesions develop elevated total body fat without any change in body weight when their hyperphagia is prevented. In part this effect seems to

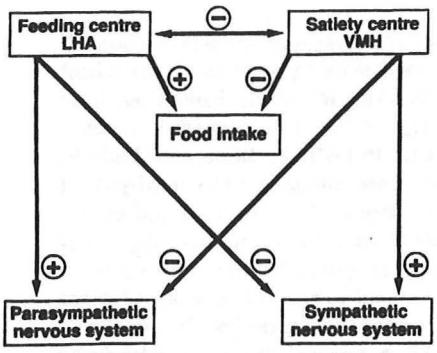


Figure 13 Scheme of the 'dual-centre' hypothesis for the regulation of food intake and the autonomic nervous system. LHA: lateral hypothalamic area; VMH: ventromedial hypothalamic nucleus. For references, see text.

be related to an impairment in thermogenesis. Metabolic changes observed in VMH-lesioned animals are analogous to those seen in the genetically ob/ob and db/db mice.

Experimental destruction of the LHA is followed by reduction in feeding, drinking and loss of body weight(58,59). The LHA-lesioned animals exhibited an increased activity of the sympathetic nervous system (60,61). Thermogenesis is greatly increased in animals with destruction of the LHA and plasma insulin levels are low. As would the expected catecholamine levels are increased and there is an increased turnover of noradrenaline in white adipose tissue, the heart and pancreas (60). Animals with LHA lesions show a marked increased in their motor activity which contributes to their significant weight loss.

Neurotransmitters in Obesity

Neuropeptide Y is produced in several areas of the brain including the hypothalamus. Neuropeptide Y stimulates food intake, decreases thermogenesis, and increases plasma insulin and corticosterone levels making it a potential obesity promoting neurohormone (62-65). Injection of neuropeptide Y into the intracerebroventricular (ICV) increased appetite even in satiated animals. This neurotransmitter also has a profound metabolic effect by increasing insulin levels and inducing insulin resistance. In a recent study, the role of neuropeptide Y was studied in ob homozygous mice who were given recombinant leptin (65). Injection of human leptin into ob/ob mice lead to a near normalization of their body weight. Measurement of neuropeptide Y mRNA in the area of the arcuate nucleus revealed a marked reduction in the expression of neuropeptide Y, following thirty days of leptin administration Fig 14. Injections of leptin into db/db/mice did not produce weight loss or reduce the hyperinsulinemia. When leptin mRNA was measured in the hypothalamus of these animals there was no significant change between the control and the leptin treated db homozygotes. Studies of radioactive leptin binding to hypothalamic plasma membranes showed that there was no differences between normal ob/ob mice and db/db mice. These findings suggests that the inhibition of neuropeptide Y synthesis and release may be one mechanism by which leptin corrects the mutant phenotype of ob/ob mice. In db/db obese mice, the truncated intracellular domain of the OB-R may impede or prevent leptin-induced signaling to inhibit neuropeptide Y synthesis and release. The continued production of this neuropeptide may be responsible for the obesity of the db/db mouse. As yet there are no studies to support

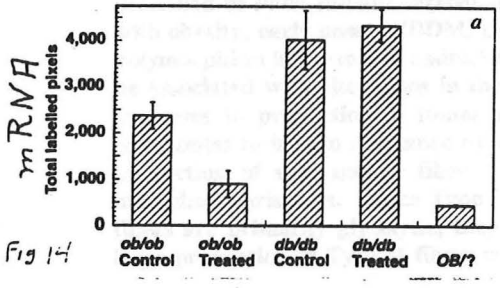


Fig 14

this speculation.

Recently a role for glucagon-like peptide-1 (GLP-1) as a powerful inhibitor of feeding in fasted rats has been proposed(66). GLP-1 when injected intracerebroventricular (ICV) localizes exclusively in the paraventricular nucleus of the hypothalamus and the central nucleus of the amygdala. ICV injection of the specific GLP-1 receptor antagonist, exendin blocked the inhibitory effect of GLP-1 on food intake. Exendin alone had no influence on fasting-induced feeding, but more than doubled food intake in satiated rats, and augmented the feeding response to the appetite stimulator neuropeptide Y. Localization of ICV GLP-1 to the paraventricular nucleus of the hypothalamus was inhibited when exendin was administered. These findings suggests that centrally expressed GLP-1 is a new physiological mediator of satiety. It is of interest that the neuropeptide Y and GLP-1 have antagonistic physiological activities and appear to balance each other. However when GLP-1 was administered to rats during a 72 hour period there was no change in the level of neuropeptide Y mRNA in the hypothalamus. This suggests that GLP-1 does not act directly by altering hypothalamic neuropeptide Y synthesis. However the increase in food intake following blockade of GLP-1 receptors by exendin and the augmented neuropeptide Y response with co-administration of exendin supports an important physiological role for central GLP-1 in the regulation of feeding. Therefore, GLP-1 may be a new physiological regulator and a central satiety factor which has important physiological activity (65).

SUMMARY

Evidence clearly indicates that genetics plays a major role in obesity and in the regions of the body in which excess fat accumulates. Comparisons of monozygotic twins reared together with those reared apart indicate that heredity may account for 70% of the similarity in body mass index (BMI). Studies of monozygotic twins responses to overfeeding and to exercise while eating a basal diet clearly indicate that there are major hereditary influences on efficiency of fuel utilization and energy expenditure. Although the genetic factors that control energy balance are not completely understood, there are some recent insights. The discovery of a new beta, adrenergic receptor that regulates thermogenesis in brown fat and in white adipose tissue, particularly in the visceral area appears to be important in energy dissipation. A missense mutation in the beta, receptor has been described in Pima Indians, French and Finnish populations and appears to be associated with obesity, early onset NIDDM, and insulin resistance. A recently discovered polymorphism in the mitochondrial uncoupling protein (UCP), in preliminary studies, may be associated with alterations in the basal metabolic rate that can contribute to obesity. Increases in production of tumor necrosis factor-alpha in fat tissue of obese subjects contributes to insulin resistance by its effects on the insulin receptor. Differences in the proportion of slow muscle fibers (Type I) to fast muscle fibers (Type II) show wide individual variations. Since Type I fibers preferentially utilize fatty acids and Type II fibers are primarily glycolytic, they have different efficiencies for energy utilization. A large proportion of Type II fibers would favor fat accumulation.

The recent application of molecular biology to genetic mouse models of obesity are providing almost daily insights. As shown in Fig 15. (see next page) Fat cells produce a peptide hormone called leptin or the OB-protein which interacts with a hypothalamic OB-receptor (OB-R) to apparently decrease the secretion of neuropeptide Y, an appetite stimulating peptide, and increase the production of a satiety factor, glucagon-like-peptide-1

providing almost daily insights. As shown in Fig 15. (see next page) Fat cells produce a peptide hormone called leptin or the OB-protein which interacts with a hypothalamic OB-receptor (OB-R) to apparently decrease the secretion of neuropeptide Y, an appetite stimulating peptide, and increase the production of a satiety factor, glucagon-like-peptide-1 (GLP-1) and perhaps other satiety factors. Activation of the nuclei in the lateral hypothalamic area (LAH) stimulates the sympathetic nervous system with catecholamine release. Activation of the beta$_3$ adrenergic receptors on fat and other cells increases metabolic rate and energy dissipation. This oversimplified model has relevance for certain mouse models of obesity where mutations in the OB gene and the OB-R gene have been recently described. What roles these proteins play in human obesity is not clear, although research in this area is extremely active. One can predict that the causes for obesity in humans is heterogeneous, but some of those newly discovered proteins may play a significant role.

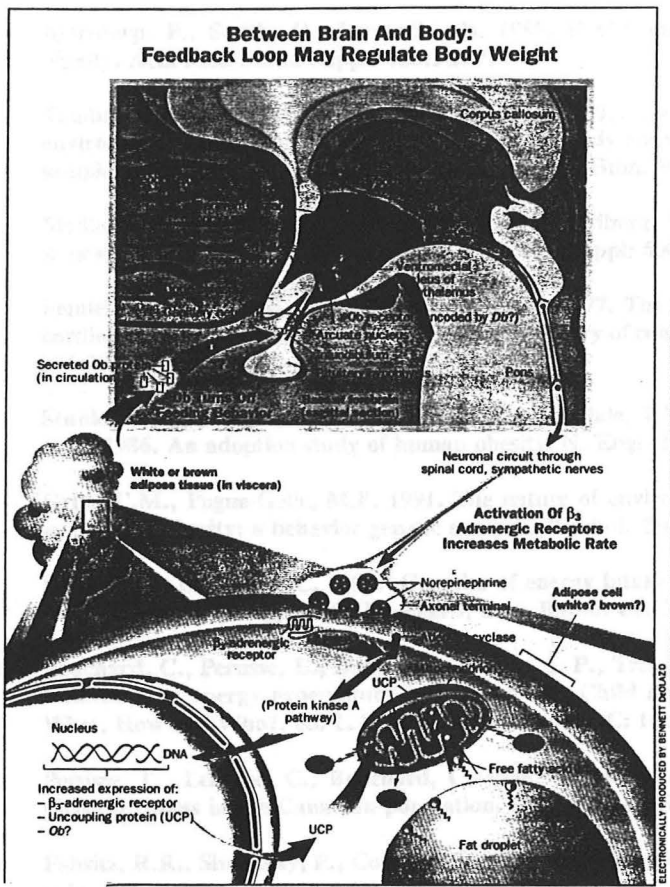


Diagram illustrating the hypothetical roles of Ob protein and the β_3-adrenergic receptor

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