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ANIMAL MODELS OF OBESITY: Genetic Aspects

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INTRODUCTION

Since 1905 when Cuenot published his observations that mice with yellow coat color became obese (41), animal models have been used to study the metabolic and behavioral changes that lead to obesity, and the results have been applied to the syndrome of human obesity. Genetically determined obesity was first described in the yellow mouse, and in 1940, Hetherington & Ranson published their first report on an experimentally produced obesity (91), which was obtained by electrolytically lesioning the ventromedial hypothalamus of the rat. Since then, a large number of new models of both genetic and experimentally produced obesity have been described, and a rich

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literature of studies comparing the various forms of these obesities has been published. Overall, these investigations have yielded an immense amount of information on the metabolic derangements and altered patterns of behavior that accompany the obese syndrome. Several excellent and comprehensive major reviews detailing this literature are available (5, 17, 20, 21, 90, 170). Table 1 provides a summation of a variety of characteristics that have been identified as part of the obese syndrome as it occurs in autosomal recessive animal models of obesity, which are those that have been the most studied to date. Despite this immense amount of information [the most recent and most comprehensive review by Bray, York & Fisler (21), although not exhaustive, lists 689 references] and the consideration of many testable hypotheses as to the underlying causes/mechanisms leading to obesity, in no case have the sequelae of events, leading from presence of a known gene or a specific experimental manipulation to development of the full blown obese syndrome, been thoroughly and conclusively described. In one instance, hypothalamic obesity produced by lesioning of the ventromedial region has been shown to be dependent upon intact neural pathways to the pancreas, since implantation of islets prevents development of obesity in lesioned rats (101). However, when knife cuts were used to isolate the hypothalamus, obesity developed in the absence of hyperinsulinemia (21), which suggests that this pathway is not totally understood.

In this review we focus attention on recent studies that utilize cellular and molecular genetic methodology in attempts to isolate and identify genes that are reasonable candidates to be syntenic with the particular gene loci, e.g. *db, ob, fa,* known to result in genetically determined obesity in laboratory rodents. The murine models of obesity are most often utilized in molecular genetic studies, because the mutant alleles that result in obese syndromes are carried on highly inbred lines, and in some cases the mutant gene has been mapped to a specific chromosome. For example, it is known that *ob* is carried on murine chromosome *6, db* on chromosome 4, and A^y on chromosome 2. In contrast, to date, no rat mutant allele has been reported to be mapped to a specific chromosome.

Several laboratories are mapping and attempting to isolate the *ob* and *db* genes from obese mice. For example, in a brief note in the 1988 *Mouse News Letter,* Friedman and his colleagues (72) reported that restriction fragment length polymorphisms (RFLPs) had been produced indicating that the *met* oncogene was located 3 centimorgans from the *ob* gene on chromosome 6 and that the *db* gene on chromosome 4 was flanked by the interferon α and Lck loci. These investigators have recently published a molecular map around the *db* locus (6a). The map is derived from RFLP analyses of the progeny of an intraspecific backcross between C57B1/6J *dbdb* and B6D2F1 *db*/+ mice, as well as the obese progeny of an interspecific cross between B6D2F1 *db*/+ and

Table 1 Selected abnormalities present in autosomal recessive rodent models of obesity[a]

Measured variable	Zucker *fafa* rat	*WDF fafa* rat	*obob* mouse	*dbdb* mouse	References
Hyperphagia	+	+	+	+	186/100/20/20
Lean body mass	−		−		156/—/128/—
Protein deposition	−		−		53/—/29/—
Urinary 3-methylhistidine	−				53/—/—/—
Glucose intolerance	+	+	+	+	197/100/40/20
Sympathetic activity	−		−		94/—/120/—
Parasympathetic activity	+				163/—/—/—
Thermogenic capacity	−		−	−	185/—/20/20
Testicular size	−		−		43/—/190/—
Response to female pheromones	−				89/—/—/—
Normalization by adrenalectomy	+	+	+	+	217/202/167/21
Hyperinsulinemia	+	+	+	transient	219/100/48/37
Hyperglycemia	−	+	+	+	197/100/48/99
Hypertryglyceridemia	++	+	nd	+	219/100/56/200
Insulin/glucagon ratio	+		−	var	9/—/63/159
Corticosterone turnover	+		+	+	216/—/49/166
Circulating estrogen	−				
Thyrotropin concentration	nd		−		214/—/215/—
Circulating thyroid hormone	−		−	−	54/—/213/116
WAT mass	+	+	+	+	111/unpubl. data/108
WAT hyperplastic	+	+	+	+	111/unpubl. data/108
WAT hypertrophic	+	+	+	+	111/unpubl. data/108
WAT lipoprotein lipase	+	+	+		86/unpubl. data/44/—
WAT lipogenesis	+		+	+	77/—/104/211
Basal lipolysis	+		+	+	103/—/150/184
Isolated WAT insulin binding	+		−		42/—/69/—
BAT mass	+		+		185/—/118/—
BAT lipoprotein lipase	nd				96/—/—/—
GDP-binding/mitochondrial	−		−	−	133/—/93/81
Pancreatic islet hypertrophy	+	+	+	+	219/100/34/20
Pancreatic islet hyperplasia	+		+	+	219/—/34/20
Pancreatic islet glucagon release	−	−	+	+	22/172/14/20
Pancreatic somatostatin	+		−	−	174/—/45/151
Pancreatic somatostatin release	+	+	nd	nd	172/172/15/15
Hepatic lipogenesis	+		+	+	77/—/104/211
Hepatic ketogenesis	−		−	−	196/—/183/26
Hepatic Na+/K+ ATPase	unaltered		−	−	20/—/213/20
f-2,6 biphosphate	++		+		98/—/31/—
g-6PDH activity	+	+			16/189/—/—
Fatty acid desaturase	+		+		203/—/57/—
Hepatic insulin resistance		+	+		—/189/177/—

Table 1 *(Continued)*

Measured variable	Zucker *fafa* rat	*WDF fafa* rat	*obob* mouse	*dbdb* mouse	References
Isolated hepatic insulin binding	+		-		155/—/78/—
Hepatic insulin receptors	var		–	–	130/—/148/178
Muscle protein	–		–		32/—/20/—
Muscle DNA	–				32/—/—/—
Muscle RNA	–				32/—/—/—
Muscle lipoprotein lipase	nd				205/—/44/—
Muscle protein synthesis	–		–		158/—/198/—
In vitro insulin resistance	+		+		105/—/69/—
Isolated muscle insulin binding	+		–		39/—/127/—
Skeletal muscle glucose utilization	–				152/—/—/—
Muscle insulin receptors	–		–		152/—/127/—
CNS insulin content	–				8/—/—/—
CNS insulin binding	–				58/59/—/—
CFS insulin content	+	+			21/59/—/—
Gonadotropin secretion	-				165/—/190/—
Hypothalamic somatostatin secretion	+.		nd	nd	174/—/15/15
Growth hormone secretion	–			–	137/—/124/176
Somatomedin activity	–				138/—/—/—
Cholecystokinin activity	+		nd		141/—/169/—
Catecholamines/adrenergic system	–		var		164/—/21/—
Dopaminergic system	–		var		164/—/21/—
Serontonergic system			+		164/—/74/—
Opioid activity	+		var		134/—/136/—

[a] The abnormalities listed illustrate the range of variables that have been measured in these obese animal models not exhaustive. Not more than one reference is provided per abnormality per model. Thus, this Table is designed to primarily an entrée into the literature.

B6 spretus F1 *db*/+ mice. As these efforts to clone the mouse obesity mutants prove successful, it should then be possible to link the primary gene defect to development of the obese syndrome in one or more animal models. Much less data have been published regarding genetic and physical mapping of rat chromosomes, although at least one group of investigators is now mapping rat chromosomes (192). Identification of chromosomal homologies between mouse and rat, such as that reported by Szpirer et al (192) for mouse chromosome 4 (locus of *db*) and rat chromosome 5, should be invaluable to investigators interested in localizing rat obesity genes.

SPECIFIC CANDIDATES FOR THE PRIMARY GENETIC LESION

A number of studies have investigated specific candidate genes to determine if they could be the locus of the primary lesion for one of the obese mutations. Most often, these studies focus on levels of gene expression in specific tissues

where known metabolic/physiologic alterations occur in obese animals. In some instances, investigations have provided a definitive answer as to whether or not the gene in question could be one of the obese mutations through mapping of the candidate gene to a specific chromosome.

Candidate Genes and Gene Expression Related to Central Nervous System Abnormalities

Several investigators have proposed that the defect that leads to obesity in *obob* and *dbdb* mice and the *fafa* rat lies primarily within the central nervous system and initiates a series of events that include hyperphagia, hyperinsulinemia, changes in both parasympathetic and sympathetic outflow, and altered regulation of the hypothalamohypophysial-adrenal axis (87). Such alterations could result in structural abnormalities in hypothalamic nuclei known to be involved in control of glycemia, insulinemia, and circadian corticosterone secretion (18). The evidence and arguments marshalled in support of this hypothesis are extensively discussed in the recent review by Bray et al (21). Numerous studies (24, 64, 209, 216) have shown that most of the metabolic defects of the genetically obese Zucker rat are reversed totally or in part by adrenalectomy and are restored by administration of glucocorticoids (68). Many investigators who have addressed the question of the presence of a central defect in the genetically obese Zucker rat by assessing the concentration, binding, and turnover of various neuropeptides and monoamines in hypothalamic regions involved in the regulation of feeding behavior and energy balance have found genotype differences (11, 46, 62, 140, 164). In addition, several of these neuropeptides associated with feeding behavior are altered in obese *obob* and *dbdb* mice but remain unchanged in their lean littermates (15, 83, 135). While gene expression studies are not yet available for the CNS monaminergic systems, several such studies have evaluated neuropeptides. These are summarized below.

NEUROPEPTIDE Y IN THE ZUCKER RAT *(fafa)* Levels of Neuropeptide Y (NPY), a 36 amino acid neuropeptide known to be a potent regulator of feeding behavior in rats (181, 182), were reported to be higher in the hypothalami of Zucker *fafa* rats than in the hypothalami of their lean littermates (199). Sanacora et al (168) demonstrated that mRNA concentrations for preproneuropeptide Y are higher in the hypothalami of obese Zucker rats than in those of lean Zucker rats and that these increased levels are localized to the arcuate nucleus. Fibers projecting from the arcuate nucleus terminate in the paraventricular nucleus (PVN) of the hypothalamus (7), and injection of NPY into the PVN of lean rats produces hyperphagia and obesity similar to that seen in obese Zucker rats (182). Furthermore, Sanacora et al (168) indicated that the magnitude of the increase in NPY expression in the obese Zucker rat

is greater than that seen for other neuropeptide alterations such as beta-endorphin (139), neurotensin (11), cholecystokinin (61), metenkephalin (140), somatostatin (46), and bombesin (146). In addition, while food-deprived lean Zucker rats showed more than a two-fold increase in NPY expression, obese animals responded to food deprivation with a modest (30%) increase, which was not statistically significant. These data suggested that NPY gene expression is already set at an abnormally high level in the obese rats, as if they were in a constant state of food deprivation. Sanacora et al (168) also proposed that both insulin and adrenal glucocorticoids may regulate the expression of the preproneuropeptide Y gene, but they did not propose that this gene could be the site of the primary genetic lesion, that is, the *fa* gene. Since expression of the NPY gene was altered in streptozotocin-produced diabetic rats, insulin is probably involved in its regulation (208). Because dexamethasone treatment enhanced NPY levels in rat hypothalamic neurons (38) and preproneuropeptide Y mRNA levels in an adrenal-derived cell line in culture (92), glucocorticoids are also likely candidates as regulators of preproneuropeptide Y gene expression. Burlet and co-workers (23) reported that obese Zucker rats show high levels of hypothalamic corticotrophin-releasing factor (CRF) and that NPY containing neurons of the PVN also take up monoclonal antibody to CRF. Microinjection of NPY into the PVN activates the pituitary-adrenal axis in the rat (204). Thus, a case can be made that NPY is directly involved in the hyperphagic response and subsequent enhanced fat accumulation and obesity in the obese Zucker rat. However, since other endocrine systems, e.g. the pancreas and the adrenal, may be involved in regulating the rate of transcription/translation of this gene, it is unlikely to be the locus of the primary genetic defect.

OPIOID PEPTIDES IN OBESE-HYPERGLYCEMIC *(obob)* AND DIABETIC *(dbdb)* MICE Obese (*ob*) and diabetes (*db*) are autosomal recessive mutations that map to different chromosomes in the mouse but cause similar if not identical diabetes-obesity syndromes when maintained on the same inbred background (34). The mechanism underlying the differences in expression of these genes when present on different backgrounds, i.e. C57Bl/KsJ or C57Bl/6J, is unknown as are the primary genetic lesions (194). A primary defect involved in synthesis of G proteins has been ruled out for *ob* (6; see below) as has the involvement of autoantibodies in *db* (125). The only comparative data that distinguish these two mutations from each other are the results of parabiosis experiments. These indicated an inability to respond to a satiety signal in *dbdb* mice, and failure to produce a satiety signal in *obob* mice (33, 36). Both findings suggested a lesion in the central nervous system (CNS). Impaired control of reproductive function mirroring impaired function of multiple pituitary cell types has been reported for *obob* mice (191); no such

abnormality was seen in *dbdb* mice (112). These findings are compatible with an hypothesis that the primary lesion in *dbdb* directly affects some part of the CNS, whereas a different lesion in *obob* mice affects anterior pituitary cells and perhaps other tissues outside the brain (194). To test this hypothesis, Timmers et al (194) measured levels of several immunoreactive neuropeptides, i.e. beta-endorphin, met-enkephalin, leu-enkephalin, and galanin in pituitary and pancreas of *obob* and *dbdb* mice. These neuropeptides were selected on the premise that *obob* mice would have low tissue content of a neuropeptide that normally inhibits tonically the rate of insulin secretion and that might also modulate secretion from the anterior pituitary, whereas the *dbdb,* with a different type of CNS lesion, would not share the same abnormality in tissue content. Their data show similar alterations in pancreatic neuropeptide content in male B6 *obob* and *dbdb* mice when compared to lean controls, which suggests that these alterations are distal components of the convergent metabolic syndromes produced by each mutation and thus do not reflect functioning of the gene carrying the primary genetic lesion. In light of the results on neuropeptide Y in the Zucker rat (168), an investigation of this neuromodulator in *obob* and *dbdb* mice appears warranted.

CHOLECYSTOKININ IN OBESE-HYPERGLYCEMIC *(obob)* MICE Cholecystokinin (CCK) is an intestinal peptide hormone that also occurs in relatively high levels in brain (145) and acts as a satiety signal (75). CCK has been considered as a potential site of the genetic defect in *obob* mice, since at least one group of investigators (188) reported extremely low brain levels in *obob* mice. However, a recent study by Friedman and co-workers (73) ruled out the CCK gene as the site of the primary lesion in *obob* mice. These investigators, using Southern blot analysis of Chinese hamster × mouse somatic cell hybridization and recombinant inbred and interspecific backcross analysis, localized the CCK gene to murine chromosome 9 (73). In addition to *ob,* other mutant alleles that produce obesity in the mouse, i.e., *db, tubby,* A^y, do not map to chromosome 9. These investigators also found no consistent differences between *obob* mice and their lean controls in levels of expression of CCK mRNA in cerebral cortex.

GROWTH HORMONE IN THE ZUCKER *(fafa)* RAT Zucker & Zucker (220) noted that rats homozygous for the *fa* gene were stunted in their linear growth, and since then other investigators have established that pituitary secretion of growth hormone (GH) is impaired in the *fafa* rat (60, 137). Ahmad et al (1) using slot-blot and cytoplasmic dot-blot analyses on pituitaries from 14-week-old male and female lean and obese Zucker rats showed that levels of GH mRNA were lower in obese than in lean rats. The fact that no differences were found between preparations from obese and lean rats with respect to prolactin

mRNA levels seems to rule out the possibility of a generalized depression in anterior pituitary activity. Furthermore, since no differences were found in DNA content between pituitaries from obese and lean rats, it is unlikely that the decreased GH mRNA is due to a general decrease in the number of pituitary cells. Although the same general depression in GH gene expression was observed in males and females, the data from females were much more variable, suggesting that gonadal steroids may influence this expression system. In a subsequent study, Ahmad et al (2) investigated pituitary GH mRNA levels in obese and lean Zucker rats during development at 3, 5, 9, and 11 weeks of age. Their data showed no differences between preparations from obese and lean rats at 3 weeks of age, but by 5 weeks and thereafter, lean rats had more GH mRNA than did obese rats. Prolactin and glyceraldehyde-3-phosphate dehydrogenase (a constitutive enzyme used as an internal control) mRNA levels showed no differences between obese and lean rats. These authors concluded that the primary defect caused by the *fa* gene is not an abnormality in GH synthesis, since other changes, e.g. hyperinsulinemia and enhanced fat deposition, are already present in 3-week-old obese rats (153). Evidence that increased pancreatic insulin secretion occurs around 2 weeks of age (19), hyperinsulinemia develops in the obese Zucker rat around 3 weeks of age (201), and that insulin suppresses GH mRNA levels in pituitary cells in vitro (210), supports the viewpoint that insulin may be involved in the regulation of GH gene expression.

Candidate Genes and Gene Expression Related to Peripheral Tissue Abnormalities

Several investigators have hypothesized that the primary lesion(s) that leads to genetic obesity is located in peripheral tissues rather than in the central nervous system. Greenwood et al (84) have proposed that the lesion resides in adipose tissue, specifically in the regulation of the "gatekeeper" enzyme lipoprotein lipase, which is responsible for the breakdown of circulating tryglyceride to free fatty acids and glycerol immediately prior to entry across the adipocyte plasma membrane. Other investigators (201) have suggested that abnormal pancreatic function leading to hyperinsulinemia is the initial event that produces the obese syndrome in genetic obesity, as seems to be the case in hypothalamic obesity (101), although it has now been shown by Chan & Stern (25) that preventing hyperinsulinemia in the Zucker rat does not prevent the obesity. We proposed that the genetic defect for the development of obesity in rodent models lies in a failure of regulation of adipocyte differentiation and/or proliferation, this failure of regulation leads to increased fat cell size and number, thereby creating a demand for substrate, and subsequently leads to the development of hyperphagia and hyperinsulinemia (110, 186). However, in vitro studies of preadipoblast cultures derived from

Zucker rats appear to rule out a primary proliferative defect in adipose tissue (107). Goldstein & Johnson (79, 80) also suggested that a basic cellular defect involving protein turnover in more than a single cell type may be the locus of the primary lesion produced by the *fa* gene.

ADIPSIN IN OBESE-HYPERGLYCEMIC *(obob)* AND DIABETIC *(dbdb)* MICE AND THE ZUCKER RAT *(fafa)* Because adipose tissue is fundamentally altered in obesity, one fruitful approach to understanding the aberrant biological functions that lead to obesity has been exploration of the processes of adipocyte growth and differentiation in cell culture systems. Using a cell line that differentiates into cells that morphologically and functionally resemble adipocytes, Green & Kehinde (82) and others (3) have been able to define rather precisely many specific steps in the process of adipocyte differentiation and to clone several genes that are activated during the differentiation process (179).

One of these differentiation marker genes codes for adipsin, a serine protease with complement factor D activity (162), which is synthesized and secreted by adipose tissue. Adipsin mRNA and circulating adipsin protein are greatly reduced in adult *obob, dbdb,* and MSG-treated obese mice (65) and also in obese Zucker rats (109). Further, Platt and colleagues (154) have shown that a marker gene linked to the adipsin promotor sequence is down-regulated in transgenic mice made obese by the *ob* gene or by MSG treatment. Thus, it seems reasonable to hypothesize that the adipsin gene may be closely related to the primary genetic defect in these genetically obese animal models. Two studies address the question of whether or not altered adipsin mRNA expression occurs prior to other events related to the onset of the obese syndrome. Lowell and co-workers (131) reported that circulating adipsin levels were 30% lower in 2-week-old *dbdb* mice than in their lean littermates. These investigators used mice in which the *db* gene was linked to the *misty* gene for coat color, which allowed easy identification of young obese mice prior to other visual evidence of the obese phenotype. In another study, Dugail and colleagues (52) reported that both adipsin mRNA and circulating adipsin protein were present in 10- and 15-day-old obese *dbdb* mice in amounts similar to those present in lean controls, but that by 30 days of age, levels of both were much lower (serum adipsin by 65%) in obese *dbdb* mice than in their lean littermates. In this study, inguinal fat was surgically removed for RNA isolation, and blood samples were taken from 10-day-old mice that were then allowed to recover and were later identified visually as lean or obese. Data from this study also showed that inguinal fat depot weights were 66% higher in preobese mice than in their lean littermates at 10 days of age, but that no hyperinsulinemia was evident at this age. Others (126) have reported that preobese *dbdb* mice have slightly increased fat stores

at 12 days of age. Since these groups of investigators used the same anti-adipsin antibody for detection of circulating adipsin protein, it is difficult to reconcile these contradictory results, other than on the basis of differences in the animals themselves or improper identification of phenotypes in the groups that were compared. In any case, the question of whether or not altered adipsin expression is a candidate for the primary genetic lesion in this obese animal model has been set to rest by Spiegelman and colleagues (180) who showed that the adipsin gene is located on murine chromosome 10 and thus cannot be identical to either the *ob* gene on chromosome 6 or the *db* gene on chromosome 4. Lowell et al (131) also measured adipsin mRNA during development of obesity that was due to MSG-induced hypothalamic damage and found it was lower in preobese mice than in controls at 2 weeks of age. This deficiency increased with age and was specific in that neither glycerophosphate dehydrogenase nor aP2 mRNAs were changed with MSG treatment.

Given that the adipsin gene is not syntenic with either *ob* (chromosome 6) or *db* (chromosome 4), the fact that adipsin expression is greatly diminished in the homozygous state for both of these mutations led Platt et al to suggest that some regulatory mechanism that influences adipsin expression must be defective (154). Platt and co-workers (154) produced transgenic mouse strains in which 950 base pairs from the 5' flanking region of the adipsin gene were linked with a bacterial reporter gene, chloramphenicol acetyltransferase (CAT). In some cases a viral enhancer gene sequence was also included in the transgenic construct. CAT activity was expressed in adipose tissue of the transgenic mice and was severely reduced (90–95%) in the transgenic progeny homozygous for *db*. With no enhancer sequence present, the adipsin promoter responded to the homozygous obesity gene with a reduction in CAT expression in adipose tissue only. With the enhancer sequence (AKR614 virus) present, CAT expression occurred in tissues other than adipose tissue, e.g. spleen, thymus, salivary gland, brain, and was reduced in these tissues in the presence of homozygous *dbdb,* thus indicating that signal transduction from the *db* gene to the adipsin promoter can operate in a variety of tissues. In studies carried out in 3T3-F442A preadipocytes undergoing adipose conversion in cell culture, Wilkison and co-workers (207) described nuclear factors that interact with sections of the adipsin promoter gene sequence in regulating expression of the CAT reporter during differentiation. Although the specific factors involved in this regulation have yet to be identified, it seems clear that the regulatory effects are exerted at the transcriptional step. Both positive and negative effects have been demonstrated, and it is certainly possible that one or more of these factors may act to suppress adipsin gene expression (207).

Spiegelman et al (180) have also conclusively demonstrated that adipsin expression in vivo is regulated, at least in part, by adrenal glucocorticoids in

both *ob* and *db* mice and in obese Zucker rats, which also have diminished levels of adipsin mRNA and circulating adipsin protein (109). These data collectively lead to the conclusion that expression of the adipsin gene is subject to a regulatory pathway that is influenced by several different genetic loci associated with the development of obesity, and that glucocorticoids are involved at some level in this regulatory pathway. However, the effect of glucocorticoid on adipsin gene expression does not appear to be direct, since it has not been possible to demonstrate effects of glucocorticoids on adipsin mRNA levels in adipocyte cultures (180). To date, studies have not revealed a glucocorticoid regulatory sequence element in the first 500 base pairs upstream of the transcription site for the mouse adipsin gene (142).

Another intriguing aspect of the adipsin story is the fact that adipsin shares 70% sequence homology with complement factor D and has been shown to have factor D activity (162). A recent study in *dbdb* mice by Montgomery et al (143) established that this obese mutant has an immunodeficiency expressed before the onset of overt diabetes. This deficiency was shown to affect both cellular and humoral immune responses; thus *dbdb* mice placed on restricted feeding to retard development of diabetes had fewer T lymphocytes and lymphocytes that agglutinated sheep red blood cells at lower serum dilutions than did lean control mice. Whether or not this immunodeficiency is related to the reduced expression of adipsin *dbdb* mice requires further investigation.

CALCITONIN IN THE ZUCKER *(fafa)* RAT Calcitonin (CT), a hormone involved in regulation of calcium metabolism, is synthesized and secreted by the C cells of the thyroid gland. Obese Zucker rats have higher than normal levels of circulating CT as early as 10 weeks of age, as well as more C cells and higher CT content in the thyroid than do their lean littermates (66, 135). Segond and colleagues (171) measured both CT mRNA and the translatable protein product of CT mRNA in thyroid tissue derived from obese and lean Zucker rats at several ages. Both CT mRNA and translatable activity level were 50% lower in 30-day-old obese rats than in lean rats, but in 12-week-old animals, both CT mRNA and translatable activity were increased in obese rats. These data suggest that the presence of the *fa* gene influences the relative abundance of translatable CT mRNA, perhaps as a result of changes in transcriptional activity or possibly changes in the rate of maturation of the mRNA. The changes in CT mRNA that occur with age in the obese Zucker rat apparently parallel developmental changes in plasma immunoreactive insulin (219). Since both the rat CT and insulin genes are located on chromosome 1 (195), Segond and co-workers (171) suggested that rearrangement of DNA around these two loci may be responsible for modulating both CT and insulin gene expression. When chromosomal localization of the *fa* gene has been

accomplished, it will be possible to either include or exclude these loci as potentially related to the *fa* mutation.

G PROTEINS IN OBESE-HYPERGLYCEMIC *(obob)* MICE AND ZUCKER *(fafa)* RATS Studies of metabolic effector systems in several tissues, i.e. adipose tissue, liver, and pancreas, that involve a guanine nucleotide regulatory mechanism (that is, involve G proteins) revealed abnormalities in obese animal models, including *dbdb* and *obob* mice and Zucker *fafa* rat. Electrophysiological tests have shown that integrity of ion channels may be impaired in pancreatic islets of *obob* mice (161). Islets from obese mice were characterized by a more depolarized membrane than were those in lean mice (160). Quinine, which blocks the ATP-sensitive K^+-activated ion channel, and apamin, which blocks the Ca^{2+}-activated K^+ channel, have atypical effects on islets from obese mice. These interactions suggest that the abnormal ionic events in these islets are related to altered production of a regulator, such as a second messenger or a G protein, common to both types of channels (67). Fournier and colleagues proposed that the genetic defect in obese-hyperglycemic mice is related to an intracellular element acting on the ionic events that are common to both the ATP-sensitive and Ca^{2+}-activated K^+ channels (67).

Activity of the adipose tissue membrane marker enzyme, 5'-nucleotidase, is abnormally low in the *obob* mouse compared with its lean control, but (Na^+-K^+)-ATPase is similar (12). Under conditions that maximize the activation of adenylate cyclase and its response to inhibitory ligands, both of these variables were significantly abnormal in membranes from obese mice and were related to decreased numbers of $G_i\alpha$ subunits of the GTP-binding protein. The $G_i\alpha$ subunits are thought to interact with β subunits, which are common to both G_s and G_i moieties of the GTP-binding protein, thereby regulating the balance between these two units, which, in turn, determines the activation of adenylate cyclase. This deficiency in $G_i\alpha$ subunits could explain the abnormal response of *obob* mouse adipose tissue to lipolytic hormones such as epinephrine and has led to the hypothesis that the *ob* mutation is due to a defect in the $G_i\alpha$ gene (13). However, Ashley and co-workers (6), using a cloned cDNA probe for $G_i\alpha$ with Chinese hamster-mouse somatic cell hybrids, have assigned the $G_i\alpha$ locus to murine chromosome 9 and $G_s\alpha$ to murine chromosome 2. Since neither of these regulatory components of the adenylate cyclase system is identical to the *db* or *ob* locus, which are located on murine chromosomes 4 and 6 respectively, the anomalies of structure of the α or β subunits of G proteins cannot be the primary defect in *dbdb* or *obob* mice, but rather may be secondary to hyperinsulinemia (13). These data on chromosomal localization for the G protein subunits would also seem to rule out a primary defect in this system in the pancreas.

Anomalies of G proteins have also been identified in the Zucker rat *fafa* (97). Houslay et al have shown that the $G_i\alpha$ subunit can be detected by Western blot in liver plasma membrane preparations from both lean and obese Zucker rats. However, membrane preparations from obese rat liver have no functional activity as demonstrated by the failure of low concentrations of p[NH]ppG to inhibit forskolin-stimulated adenylate cyclase activity. These authors (97) propose that this functional failure could be due either to a genetic mutation leading to a defective protein or to a posttranslational process that inactivates G_i, most likely phosphorylation. In addition, it has been shown in obese Zucker rats that G_s has a limited capacity to couple glucagon receptors to the adenylate cyclase system. The multiple defects in G proteins that are present in the Zucker *fafa* rat lead these investigators to conclude that the G protein system is unlikely to be the site of the primary genetic lesion.

GLUCOSE TRANSPORTERS IN DIABETIC *(dbdb)* MICE AND THE ZUCKER RAT *(fafa)* Insulin resistance of peripheral tissues, primarily skeletal muscle and adipose tissue, is a common feature of most genetically obese animal models and is most often attributed to defects in the insulin-mediated transport systems for glucose residing in these tissues (102, 114). The glucose transporter proteins that mediate glucose movement into muscle cells and adipocytes are prime candidates for the defect that leads to insulin resistance, and the number of transporters has been shown to vary with insulin resistance (113, 114). Recent studies have identified a family of tissue specific proteins that act as transporters, and a number of the genes that code for these proteins have been cloned (28, 144, 193). Koranyi and co-workers (122) measured tissue glucose transporter mRNA and protein levels in diaphragm, heart, skeletal muscle, and adipose tissue from 5-week-old *dbdb* mice and their lean littermates. The major glucose transporter type identified in these tissues was GLUT-4. The level of GLUT-4 mRNA in heart, diaphragm, and adipose tissue was similar for obese and lean animals but was reduced in quadriceps of obese mice. However, the levels of GLUT-4 protein were similar between obese and lean mice in all four tissues studied by immunoblotting using a polyclonal antiserum. The discrepancy between quadriceps mRNA and protein levels has been reported previously for normal rats (102). Although a previous report (27) has established that insulin resistance in the *dbdb* mouse is related to a major defect in glucose transport, the study by Koranyi et al (122) appears to rule out changes in total muscle glucose transport protein as the underlying defect. Similarly, Friedman and co-workers (71) reported that GLUT-4 levels in gastrocnemius muscles of obese Zucker rats were similar to those of lean littermates, although the insulin resistance in these obese animals has been reported to be the consequence of defective glucose trans-

port into muscle (175, 218). These data suggest that the defect in glucose transport is likely to lie in signal transduction or translocation of the transporter or the functional activity of the transporter, rather than in the amount of transporter protein in the tissues. Horton and colleagues (95) have reported that obese Zucker rat skeletal muscle has a defect in translocation of glucose transporters to the plasma membrane; they noted that the intrinsic activity of the transporters under insulin stimulation is normal. They suggested that the deficit may lie in regulation of the intracellular transport pool size or in a translocation signal and/or mechanism. It is tempting to speculate that, as in the case with adipsin, the *db* and/or the *fa* mutation, which cause severe obesity in mouse and rat respectively, affect a number of systems involved in regulatory pathways in several tissues.

In the pancreas, transmembrane glucose transport is involved in glucose-stimulated insulin secretion; the specific glucose transporter in this tissue is GLUT-2 (193). Johnson and co-workers (106) investigated the impaired glucose-stimulated insulin secretion in a partially inbred line of Zucker rats in which the males, but not the females, develop severe hyperglycemia (30). Their data demonstrate that in hyperglycemic male rats in which β cells were unresponsive to glucose stimulation, both immunoreactive GLUT-2 and GLUT-2 mRNA were substantially lower than in lean male controls. GLUT-2 protein and mRNA were normal in equally hyperglycemic female rats in which normal responsiveness of β cells to glucose stimulation was maintained. Johnson et al concluded that an unknown factor leads to decreased expression of the GLUT-2 message, which in turn reduces the capacity of the β cell to respond normally to glucose stimulation, thus resulting in uncompensated hyperglycemia (106). The factor(s) involved in this regulation remains unidentified, but acts in a tissue-specific manner, since GLUT-2 expression was unaffected in hepatocytes of the male diabetic rats.

INSULIN AND ITS RECEPTORS IN GENETICALLY OBESE MICE AND RATS

Most of the animal models of obesity exhibit hyperinsulinemia, some degree of hyperglycemia under certain conditions, peripheral insulin resistance, abnormalities in pancreatic islet morphology (20), and altered insulin receptor function (85, 178). Consequently, several studies have investigated candidate genes related to insulin metabolism in obese animal models.

Analysis of the potential role of insulin genes in these models has been complicated by the fact that both mice and rats express two nonidentical, unlinked insulin genes (129, 206). Because both of these genes are highly conserved (206), the two protein sequences for which they code may have distinct physiological functions. However, studies in both rats (76) and mice (123) with obesity/diabetes have established that the two genes are coordinately regulated. In noninsulin dependent diabetic rats, as plasma glucose

levels increased, the pancreas did not augment insulin mRNA levels (76). Further, 5-week-old *dbdb* mice have a 4-fold elevation in plasma insulin and in pancreatic proinsulin mRNA concentration that decreases as the animals age and become more glucose intolerant (149). Using a highly sensitive RNAse protection assay, investigators attempted to determine whether or not the elevated insulin production in 5-week-old *dbdb* mice was mediated by selective transcription of one of the two genes (123). Higher rates of insulin synthesis and secretion in the obese, as compared to the lean, mouse were reflected in elevated plasma insulin, elevated pancreatic insulin mRNA and lower pancreatic insulin content. The abundance of the two types of insulin mRNA, mPl-1 and mPl-2, was similar. It is unlikely that a selective defect in one of the two insulin genes is responsible for the difference in insulin synthesis that occurs when the *db* mutation is imposed on differing genetic backgrounds (123). On the other hand, genetic analysis of the effects of the *db* locus in mouse strains that differ in susceptibility to development of obesity-associated diabetes suggests that insulin-synthesizing capacity in *dbdb* mice is under polygenic control (115), as is the production of autoantibodies to islet tissue in inbred strains carrying obesity genes *db* and *ob* (212).

Studies of the levels of preproinsulin mRNA in Zucker (*fafa*) and Wistar diabetic fatty (WDF*fafa*) rats indicate that insulin genes are subject to differing regulation in these two animal models and that one model becomes frankly diabetic (WDF*fafa*) while the other (*fafa*) does not (121). Pancreatic preproinsulin mRNA along with plasma insulin increased with age to a greater extent in obese than in lean Zucker rats, suggesting that increasing fat deposition led to enhanced insulin gene expression in these animals. In 5- and 14-week-old diabetic fatty Wistar rats, no differences were found between obese and lean rats in pancreatic preproinsulin mRNA levels or in insulin content. At 5 weeks of age, the preproinsulin mRNA level in diabetic fatty Wistar rats was less than that of obese Zucker rats. Koh et al (121) suggested that some failure of insulin gene expression occurred in the diabetic fatty Wistar rat and that it could be genetically determined. If this hypothesis is correct, then it will be important to assess the effects of genetic background on insulin gene expression in these rat models of obesity.

Since insulin genes I and II have been localized to chromosome 7 in the mouse, they are ruled out as candidates for the primary genetic defect in *obob, dbdb,* and $A^y a$ mice.

In addition to anomalies in insulin biosynthesis and secretion by pancreatic islets in obese animal models, defects in insulin binding (127), insulin receptors (148, 157, 178), and phosphorylation of the insulin receptor β subunit (85, 173) have also been described. Ludwig and co-workers (132) using an insulin receptor cDNA probe (55) have demonstrated that the reduced number of hepatic insulin receptors in both *obob* and *dbdb* obese

mice cannot be explained by lower insulin receptor mRNA content or grossly altered mRNA structure. In both obese genotypes, total hepatic insulin receptor mRNA content is significantly higher than in lean controls. Restriction fragment length polymorphism analysis also failed to show any differences in the insulin receptor gene itself among the mouse genotypes studied (132). Thus, the decreased receptor number in these genetically obese, insulin-resistant animal models must be due to a posttranscriptional defect. Although the increased insulin receptor mRNA in tissue from obese animals may be secondary to enhanced glucocorticoid levels acting to increase transcription, specific translational or posttranslational events are likely to be responsible for the dissociation between enhanced mRNA levels and decreased insulin binding (132).

ISLET CELL AUTOANTIBODY PRODUCTION: RELATIONSHIP TO *ob* AND *db* GENES Autoantibodies can be raised against pancreatic islet cell cytoplasmic antigens (ICAs) in inbred mice of the C57Bl/KsJ (BKs) strain (212). This is the background strain in which the *db* mutation occurred spontaneously, and when the mutation is expressed in this strain, both male and female mice become severely obese and develop beta cell necrosis and severe hyperglycemia by 6 months of age (35). If the *db* gene is transferred to a C57Bl/6J inbred strain, the obese/diabetic syndrome is modified such that insulin resistance is present with no frank destruction of islet β cells (34). Thus, the pathology of islet β cells in *dbdb* mice appears to result from interactions between the mutant gene and the inbred strain background. Among the other background strain genes thought to influence the expression of the obese/diabetic syndrome are those at the major histocompatibility complex (MHC), which exert major influence on immunologic responsiveness to insulin (117). Other loci involved in modulation of ICA expression include one for steroid sulfatase that is present on both X and Y chromosomes but is differentially expressed in BKs (diabetes susceptible) and B6 (diabetes resistant) strains (125). Thus, when the *db* or the *ob* mutation is introduced into different background strains, the stress resulting from the obese mutant produces varying degrees of expression of ICA antibody production (212). These authors conclude that both *db* and *ob* genes influence islet cell autoantibody production, but in a way that is modulated polygenetically. Bahary et al (6a) describe a wide degree of variability in plasma glucose and insulin values in the *dbdb* progeny of the backcross they used to map the *db* locus. They conclude that the role of background strain in influencing expression of the *db* mutation is likely polygenic in nature. Delineating these genetic control mechanisms may help us understand the sequelae of events that occur during development of obese syndromes with and without expressed diabetes.

ADIPOSE TISSUE ENZYMES IN THE ZUCKER *(fafa)* RAT A number of enzymes involved in the metabolic pathways for lipogenesis, glycolysis, and gluconeogenesis are known to be altered in adipose tissue and liver of genetically obese animals, including the fatty Zucker rat. Several lipogenic enzymes are increased in adipose tissue of 7-day-old obese Zucker rats prior to onset of hyperinsulinemia (10), which suggests that changes in specific enzyme synthesis in adipose tissue are more proximate to the primary genetic lesion than are changes in insulin synthesis and secretion. Several laboratories have investigated levels of mRNA expression for some of these enzymes in efforts to determine if pretranscriptional events can explain the enhanced enzyme activities.

Glyceraldehyde-3-phosphate dehydrogenase Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) has been proposed as a regulatory enzyme of glycolysis in adipose tissue (88). Thus, its activity could influence glycolytic rates and thus the supply of substrates for lipogenesis in adipose tissue. GAPDH activity in biopsied inguinal fat was slightly higher in 7-day-old Zucker obese rat pups than in lean rat pups; by 16 days of age the difference was threefold; and by 30 days of age when the pups were weaned, the difference had become approximately tenfold (50). Levels of GAPDH mRNA in inguinal fat tissue were also higher in 16- and 30-day-old obese rats than in lean rats by approximately the same amounts as the increases seen in enzyme activity. These differences were specific to adipose tissue GAPDH, since a cDNA probe for cytochrome oxidase showed no differences in mRNA between adipose tissue samples from obese and lean rats, nor did GAPDH mRNA levels differ in liver samples from obese and lean rats. Thus, both mRNA and enzyme activity for GAPDH were elevated prior to, as well as after, the onset of hyperinsulinemia. These data suggested that a pretranscriptional event for this adipose tissue enzyme may be altered in obese Zucker rats.

Lipoprotein lipase Lipoprotein lipase (LPL) is much higher in genetically obese Zucker rats than in their lean littermates (86) and is also higher in obese than in lean humans (147). Furthermore, since elevated LPL levels are among the changes that can be detected in the obese Zucker rat at an early developmental stage and before the onset of hyperinsulinema (86), a defect associated with the synthesis/secretion of this enzyme has been postulated as a candidate for the primary genetic lesion produced by the *fa* gene. Using immunotitration, Dugail and co-workers (51) showed that increased adipose tissue LPL activity reflects increased LPL protein, with no change in specific activity; this finding suggests that either LPL synthesis was increased or LPL

degradation was diminished in this tissue. Fried and co-workers (70) have completed a study of LPL synthesis and degradation in isolated adipocytes from young lean and obese Zucker rats. Rates of LPL synthesis as measured by immunoprecipitation from cell lysates were fivefold higher in adipocytes from obese rats than from lean rats when expressed per cell; however, rates of total protein synthesis expressed per cell were also about fivefold higher in cells from obese rats than in cells from lean rats. Similarly, levels of LPL mRNA, expressed as percent of total cell RNA, were the same in lean- and obese-derived cells. Because cells from obese rats had about twice as much total RNA as cells from lean rats, they also had at least twice as much LPL mRNA. Thus, the relative rate of in vitro LPL synthesis as reflected by both mRNA and protein content was similar in cells from obese and lean rats, but the absolute rate of synthesis expressed per cell was two- to fourfold higher in obese-derived than in lean-derived adipocytes. Likewise, LPL degradation was elevated substantially in cells from obese rats. Thus, it can be concluded that turnover of LPL in obese-derived cells occurs at a higher rate than it does in lean-derived cells. However, the enhanced turnover cannot be explained by a specific alteration in LPL gene expression, since the relative rates of LPL synthesis and degradation and the LPL mRNA levels are all similar in obese- and lean-derived cells in vitro. Once again, it is likely that posttranslational control mechanisms are influencing LPL gene expression. Given that adrenalectomy in obese Zucker rats tends to normalize LPL activity and injection of glucocorticoids tends to return activity to levels seen in sham-operated obese rats (68), the glucocorticoids are likely to be involved in posttranslational modification of LPL turnover in adipocytes. S. K. Fried (personal communication) has shown that glucocorticoids in the presence of insulin decreased rates of LPL degradation in isolated human adipocytes, thus increasing LPL activity. Insulin also influenced LPL synthetic rates by enhancing total protein synthesis in adipocytes (70), but it is less clear whether or not insulin has a specific effect on LPL synthesis apart from the overall effect on total protein synthesis as measured in isolated cells. Doolittle et al (47) showed that the changes in LPL activity in adipose tissue and heart that occur in response to fasting and refeeding were modulated posttranslationally, and were likely influenced by a number of humoral factors including glucocorticoids, insulin, glucagon, and sympathetic innervation. We expect that similar posttranslational modifications exert influence on LPL activity in the obese rat. In any case, these data taken together appear to rule out a direct effect on LPL synthesis as the primary site of action of the *fa* gene. Also, we note that the LPL structural gene has been localized to chromosome 8 in both mouse and human (119), although it has not yet been mapped in the rat.

SUMMARY

Among the candidate genes that have been reviewed herein, adipsin, calcitonin, cholecystokinin, $G_i\alpha$ and G_s subunits of G proteins, insulin I and II, and lipoprotein lipase have all been mapped to specific chromosomes in mouse or rat or both. In none of these cases is the chromosomal location syntenic with murine obesity genes *db* (on chromosome 4), or *ob* (on chromosome 6). Thus, all of these genes that code for metabolic modulators that are altered in obese animals but not in lean animals can be ruled out as possible loci of the primary genetic defect, at least for the murine models of obesity. In the case of neuropeptide Y, growth hormone, glucose transporter GLUT-4, the insulin receptor, and glyceraldehyde-3-phosphate dehydrogenase, chromosomal mapping has not yet been reported. However, in each of these cases, the evidence available strongly argues against any one of these physiologic modulators as the likely site of the primary defect for any one of the obesity mutations. Rather, in all of these cases, regardless of whether or not the gene has been mapped, the evidence suggests that posttranscriptional and/or post-translational processes are involved in bringing about the specific alterations in level or activity of the protein product that is seen in the obese animal. Often hormonal regulation is invoked as a possible explanation for the changes observed in gene expression. The hormones most commonly identified as having a mediating effect on the particular metabolic pathways involved are insulin and/or the adrenal glucocorticoids. Since in each of the obese mutants, circulating amounts of these hormones are elevated, severely so in the case of insulin, it would not be surprising to find that they influence the levels and activities of many protein products involved in a variety of central nervous system and peripheral metabolic pathways. Glucocorticoids are known to exert direct effects on gene expression; however, with respect to adipsin gene expression, a direct effect has not been found (142). Furthermore, insulin itself has been considered as a candidate for the genetic lesion in these animals and has been ruled out by chromosomal localization. Thus, while it may certainly prove to be the case that both insulin and glucocorticoids affect these systems in some way, their effects appear to be indirect.

The work by Platt and colleagues (154) in transgenic mice provides the first evidence of signal transduction between an obese mutant allele and the promoter sequence for a gene that shows significantly altered expression in the obese animal. Future studies should reveal how obese mutations exert their influence on the expression of many other structural genes in a variety of tissues that undergo significant alterations in obesity. Current data suggest that the mutant allele, in this case *db,* may code for a regulatory element that can interact with promoter sequences to alter gene expression in a variety of tissues. In 1982, we proposed that the *fa* gene may exert its influence on a

fundamental cellular regulatory function pleiotropically (201). Given the information now available on numerous candidate genes that have altered levels of expression in tissues ranging from specific hypothalamic brain regions to liver, pancreas, and adipose tissue, and given the ability to construct appropriate vectors for production of transgenic animals, investigators interested in understanding genetic obesity will be able to test similar hypotheses.

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