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# Transgenic and knockout rodents: Novel insights into mechanisms of body weight regulation

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*Transgenic animals that over- or underexpress a protein of interest have been used to study obesity development, prevention, and susceptibility to diet-induced obesity such as a high-fat diet. Several transgenic models are resistant to diet-induced obesity including those that overexpress the insulin-sensitive glucose transporter, GLUT4, in adipose tissue only. In this animal there is increased adipose tissue mass but the animal maintains its insulin sensitivity. The overexpression of lipoprotein lipase (LPL) in skeletal muscle and the elimination of a protein kinase A subunit both resulted in lean and obesity resistant animals. By directing the production of the diphtheria toxin A chain to adipose tissue only the resulting animals not only had less adipose tissue mass but were resistant to MSG-induced obesity. Conversely, transgenic models with decreased brown adipose tissue or its function have all resulted in obese animals, highlighting the importance of thermoregulation in body weight maintenance. The use of transgenic technology in the field of obesity has emphasized the regional differences among fat pads as well as the dissimilarity between genders in fuel metabolism. Several transgenic models have separated obesity from insulin resistance allowing the importance of each state to be studied individually. Results using transgenic animals have re-emphasized that obesity is a polygenic disease.* (J. Nutr. Biochem. 8:702–706, 1997) © *Elsevier Science Inc. 1997*

**Keywords:** obesity; body composition; adipose tissue; appetite regulation

## Introduction

The advent in the use of transgenic animals as a method to study metabolism has provided the field of obesity research with a powerful tool. Human studies have repeatedly shown that genetics account for up to 75% of obesity.[1] The susceptibility to obesity was also noted in several rodent strains. Although the genetic defect has been elucidated in several strains such as the *ob/ob*, *db/db*, and in the agouti mouse, the applicability of these models to human obesity remains undefined. Naturally occurring genetically obese rodents typically have a single genetic alteration, whereas human obesity, or the susceptibility to it, is polygenic in nature. Creating transgenic animals allows the researcher to test specific hypotheses about the effect of a single gene or multiple genes on obesity development and what role the environment has in specific metabolic alterations.

Transgenic animals are created by the microinjection of linearized DNA into fertilized oocytes that are then implanted into pseudo-pregnant foster mothers. Typically mice are used, presumably for economic reasons, although tissue sample sizes can be limiting. The DNA injected usually contains three important components: a promoter that serves as an on/off switch for the gene of interest, the DNA sequence that codes for the protein of interest, and an indeterminant amount of poly A, which seems to play a necessary regulatory role. Often the DNA construct will also include introns that serve to increase expression levels.[2] Depending on the purpose of the transgenic experiment these DNA segments can be from different genes. Promoter sequences can confer tissue specificity and to a certain extent how often the DNA of interest will be transcribed. High-expression promoters such as B-actin can be used to drive generalized overexpression of a protein. Likewise, a promoter of a given gene product can be linked to a reporter gene such as luciferase or chloramphenicol acetyl trans-

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Received March 25, 1997; accepted August 4, 1997.



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0955-2863/97/$17.00
PII S0955-2863(97)00123-X

**Table 1** Transgenic models resistant to obesity

| Model | Body weight | %Fat | Obesity induction |
|---|---|---|---|
| GLUT4 overexpression in adipose tissue | Increased after 5 wks | Increased | Resistant |
| LPL overexpression in muscle | Similar | Lower | Resistant |
| Diphtheria expression in adipose tissue | Similar in intermediate and low expressors | Similar | Resistant |
| IGF-II overexpression | | Decreased | ND |
| Glycerol-3-phosphate dehydrogenase | Similar | Lower | ND |
| UCP in WAT | Similar | Lower but age and fat pad specific | ND |
| RIIB knock-out | | Lower | Resistant |
| MT-Growth hormone | | Lower | Prone to obesity when GH withdrawn |

ND, not done

ferase to determine which tissues express the gene product and its endogenous regulation. This approach can be modified to produce "knock-out" transgenics that fail to produce a specific protein of interest. This can be accomplished by interfering with the endogenous gene's transcription or translation process.

Thus, by simply over- or underexpressing a protein of interest transgenic animals can be used to study many aspects of metabolism, including function of a gene, tissue specificity of its expression, or gene regulation. Additionally, transgenic animals have been used to establish brown fat cell lines,[3] to decipher which receptors drugs specifically use,[4] and to discern endocrine versus paracrine effects of a given protein.[5] Transgenic models can also be used to confirm the role of suspected genetic mutations such as that seen with the agouti protein and the $B_3$-adrenergic receptor.[4,6]

## Alterations in body composition

Transgenics have been designed to study obesity development, prevention, and susceptibility to environmental signals such as a high fat diets. This paper will first describe transgenic models that resulted in altered fat mass (*Table 1*) attributable to the genetic manipulation of a specific metabolic protein. Second, transgenic mice that were expressly designed to alter either energy intake or energy expenditure will be detailed under the heading *Changes in Energy Balance*. Last, a *Fortuitous Insights* section will outline transgenic models that have resulted in serendipitous findings providing insights into either adipose tissue metabolism or modifiers of gene expression. What is most striking about transgenic research is how often the resulting phenotypes provided an unexpected finding. The original GLUT4 transgenic was an animal that overexpressed GLUT4 in adipose tissue only.[7] This mouse was created to alter glucose metabolism by increasing insulin-stimulated glucose uptake. The resulting mouse ended up with large amounts of adipose tissue and as expected, was quite insulin sensitive. This was the first time fat accumulation had been dissociated from insulin resistance providing a very powerful model to study fat tissue metabolism. From these animals it was unclear if their increased insulin sensitivity permitted the development of obesity as some would propose.[8] Interestingly, the extra adipose tissue mass resulted from an increased number of fat cells not increased fat cell size, suggesting that increased glucose may be a stimulus to increase the number of fat cells. But the surprises were not over. When these transgenics were fed a high fat diet they did not accumulate extra fat mass as the nontransgenics did, and their fat cell size remained similar to the low-fat controls.[9] These data support the hypothesis that the fate of a nutrient, in this case glucose, is dependent on the genetic background, whether the genes are from the endogenous strain or an introduced transgene. Furthermore, these data implied that obesity could potentially be prevented by expression of the appropriate gene(s).

This concept was put to test in a transgenic model that overexpressed lipoprotein lipase (LPL), specifically in muscle.[10,11] It was hypothesized that increased LPL, the enzyme that hydrolyzes circulating triglycerides into free fatty acids for tissue uptake, in muscle would result in increased fat oxidation and thus less fat storage. Indeed this was the case. Although body weights were similar, body fat decreased 17%.[11] Moreover, when these transgenics were fed a high-fat diet, fat accumulation was attenuated.

A more anatomical approach to eliminate adipose tissue was the use of a transgene in which the fat-specific promoter of the ap2 gene was linked to direct production of diphtheria toxin A chain.[12] Expression of the diphtheria protein results in the inhibition of protein synthesis and thus kills any cells that express it. Because transgenes are incorporated in to the germ line in different locations and with different copy numbers, the first generation of founders will display varying levels of transgene expression. Each founder is then bred to become its own transgenic strain. In this model, the animals that expressed high amounts of diphtheria only lived a few days. The absence of adipose tissue resulted in an inability to store dietary fat, which then accumulated in the peritoneal cavity resulting in fatal ascites. Mice with intermediate expression had similar fat pad weights until 4 months, at which time adipose tissue loss started to occur, accompanied by histologic abnormalities. Despite somewhat normal amounts of adipose tissue, these transgenics were essentially obesity resistant. When treated with monosodium glutamate (MSG), an agent which results in hypothalamic defects and subsequently obesity,

the transgenics were able to prevent the expected increase in adipose tissue mass.

Several transgenics have been created with varying levels of generalized LPL overexpression.[13–15] Transgenics that have whole-body overexpression of LPL seem to have similar body weight and fat mass.[15] It should be noted that with these promoters, LPL expression is increased only 2- to 5-fold, whereas tissue-specific expression typically results in a 4- to 100-fold increase.[10,11] With the inclusion of an intron in the LPL gene cDNA, LPL expression is further increased resulting in mice with less body fat but similar body weight as described previously.[11] Transgenic mice with more than a 100-fold increase in skeletal muscle LPL resulted in increased peroxisomes and subsequently, muscle myopathy.[10] These high-LPL-expressing animals not only had less adipose tissue but less lean tissue and subsequently died prematurely. Thus, the level of expression can result in different phenotypes. Conversely, the LPL knockout have decreased adipose tissue stores too, but die by 18 hr of chylomicronemia.[16] If these animals are bred to the muscle-only LPL overexpressors they then live a normal life span with normal amounts of adipose tissue. This suggests that LPL is critical for clearing plasma triglycerides, but not necessary for the development and maintenance of adipose tissue.

Adipose tissue lipid accumulation has also been prevented by the over expression of insulin-like growth factor-II (IGF-II).[17] In these IGF-II animals fat oxidation was increased and, based on several lines of evidence, it was speculated that the increased fat oxidation was attributable to more active brown adipose tissue (BAT). BAT is mitochondria-enriched adipose tissue that uniquely produces the uncoupling protein (UCP). UCP functions as a proton channel allowing the uncoupling of energy production from respiration. Interestingly, the diminished adipose tissue mass is further decreased as the animal ages. BAT was specifically increased in a transgenic model that overexpressed glycerol 3-phosphate dehydrogenase (G3P-DH) only in BAT by using the uncoupling protein promoter, which confers BAT specificity.[18] This G3P-DH animal resulted in more lipid being stored in BAT at the expense of white adipose tissue (WAT), which was greatly decreased despite similar body weights.[18] When UCP was expressed specifically in WAT, WAT was significantly decreased at 3 months, despite similar body weights.[19] The decrease in adipose tissue was specifically seen in subcutaneous and mesenteric fat. This decrease in adipose tissue was age and fat pad specific. At 2 months transgenics and nontransgenics were similar but as they aged the decreases in subcutaneous and mesenteric adipose tissue became apparent, especially in the females. By 1 year subcutaneous adipose tissue was barely detectable but in the females gonadal adipose tissue had increased. The male transgenics additionally had diminished renal fat pads. When this UCP-1 transgene was put into the $A^{vy}$ background, a genetically obese mouse, the transgene decreased obesity by approximately 15%. Obesity was further attenuated with age in the female transgenics only.

BAT activity was also increased in the RIIB knockout mouse. In this model the RIIB subunit of protein kinase A was knocked out resulting in increased cAMP in BAT, among other tissues, and hence, increased fat oxidation.

**Table 2** Transgenic animals prone to obesity

| Model | Body weight | %Fat |
|---|---|---|
| Antisense to glucocorticord receptor in CNS | Increased | Increased |
| Diphtheria expression in BAT | Increased after weaning | Increased |
| Decreased insulin receptor in skeletal muscle | Similar | Increased |
| Increased GLUT4 in adipose tissue | Increased | Increased |

Accompanied by a 35% increase in lipolysis in WAT, animals were lean and resistant to diet induced obesity.[20]

Transgenics have also been created with growth hormone on an inducible promoter,[21] from the metallothionien gene, which is regulated by zinc. When zinc is added to the drinking water excess growth hormone is then produced. This resulted in decreased adipose tissue. Most interesting, the withdrawal of zinc and thus the return of growth hormone to normal levels resulted in obesity, even if food intake was maintained at 80 to 90% of controls. This animal could provide a useful model for reduced obese humans whom are also prone to weight gain after fat reduction.

## Changes in energy balance

Obesity is a disease that results from energy imbalance: energy intake exceeds energy output. There have been several transgenics created to explore the role of specific proteins in maintaining energy balance (*Table 2*). The hypothalamus has long been implicated in the development of obesity and hence, is a natural brain region in which to explore the role of neuropeptides in appetite regulation. To further understand the role of NPY in energy intake, the Neuropeptide Y (NPY) knockout was created in the *ob/ob* background, a mouse that lacks the *ob* protein resulting in increased energy intake and thus obesity.[22] In *ob/ob*, NPY is elevated. When NPY was knocked out in the *ob/ob* mice, obesity was attenuated but not prevented. This decrease in weight was due to decreased food consumption as well as increased energy expenditure. The NPY knockout animals have confirmed the importance of NPY as an energy balance modulator, as well opening up the door to study the remaining neuromodulators which must also be functioning concurrently.

To explore the role of the sympathetic nervous system in obesity, the $B_3$-adrenergic receptor was knocked out.[4] Naturally occurring $B_3$ receptor mutations have been found in humans and in some populations were associated with obesity and an early onset of NIDDM. The $B_3$ knockout mice had a small increase in body fat but not enough to suggest a role for $B_3$ in obesity development. But now these mice can be used to observe the interaction of environment such as a high fat diet on this genetic background.

A transgenic model with severe muscle-specific insulin resistance (the insulin receptor was inhibited in skeletal muscle only) resulted in increased adiposity, suggesting that the insulin resistant state may result in increased adipose tissue mass.[23] The authors speculated that hyperinsulinemia

may be acting as an anabolic agent in adipose tissue. This assumes that insulin sensitivity is maintained in adipose tissue. This was not specifically measured. These transgenic animals were also hyperglycemic in the fasting state. As in the adipose tissue-GLUT4 overexpressing model, glucose may once again be acting as a stimulus of fat cell proliferation. Alternatively, glucose has been shown to inhibit lipolysis, independent of insulin.[24] This would result in increased lipid retained in the fat pad as seen in this transgenic.

Using the diphtheria toxin approach, transgenics with the ablation of brown adipose tissue (BAT) have been created.[25] BAT is thought to be essential for the maintenance of energy balance and thermogenesis. Without BAT, these mice become morbidly obese and more susceptible to diet-induced obesity.[26] The animals became hyperphagic, perhaps attributable to the lack of ability to generate heat in response to feeding, a signal that has been proposed as an appetite terminator.[27]

Transgenics were also developed to confirm that the agouti protein was responsible for the obesity noted in the $A^y$ mouse, a naturally occurring mutant that mistakenly expresses agouti in all tissues without specificity.[5] Additionally, through the use of different tissue-specific promoters, several transgenics were developed resulting in an overproduction of agouti either systemically or in specific tissues. As a result it is now known that agouti works in a paracrine fashion, although it is still under investigation which tissue is overexpressing agouti to cause the obesity noted in the $A^y$ mouse.

Transgenics that overexpress antisense RNA to the Type II glucocorticord receptor specifically in the central nervous system resulted in obesity.[28] These mice have reduced energy expenditure and thus obesity despite eating less than the nontransgenic mice.

In summary, these transgenic models suggested that increased energy expenditure, one side of the obesity equation, can result in diminished fat tissue in some mice but that other factors can attenuate this effect as noted in the $A^y$ mouse. The systems that regulate body weight and fat mass seem to be quite complex.

## Fortuitous insights

Several transgenic animals have shed light on the metabolism of adipose tissue and its potential role in obesity. The GLUT4 overexpressing transgenic indicated that glucose was a stimulus for fat cell hyperplasia. The GLUT4 knockout mouse has confirmed this.[29] The GLUT4 knockout animals have severely reduced fat tissue and are growth retarded. Additionally, if phosphoenolpyruvate carboxykinase (PEPCK) is overexpressed resulting in increased gluconeogenesis, increased body weight is also noted, although it is unknown if this reflects increased adipose tissue mass as body composition was not reported.[30] Oddly, the overexpression of Ha-ras in adipose tissue, which resulted in elevated levels of basal and insulin-stimulated glucose uptake in adipose tissue, produced an animal that had smaller fat cells and less fat tissue.[31] Because ras is involved in the insulin signaling cascade it is unclear if its' overexpression was additionally influencing other pathways.

In an effort to understand fatty acid metabolism in fat tissue, a transgenic was created that was missing the ap2 gene.[32] ap2 is a fat-specific fatty acid binding protein, the precise role of which in adipose tissue is undefined. These mice were normal in body weight and adiposity until challenged with a high-fat diet. Although both the transgenic and the non-transgenic mice gained fat mass only the nontransgenic mice became insulin resistant as estimated by fasting plasma insulin and by glucose tolerance tests. In this model, adipose tissue tumor necrosis factor-α (AT-TNF) was not elevated. AT-TNF has been associated with both obesity and insulin resistance. Thus this model would suggest that either TNF mRNA is regulated by the ap2 protein or alternatively by insulin, because the obese transgenics had no increase in plasma insulin. Most importantly, these data suggest that obesity does not necessarily have to result in insulin resistance.

Results using transgenic animals have re-emphasized that obesity is most likely a polygenic disease. Depending on the genetic background of the animal a transgene may have different effects. For example, in the B-cell glucokinase knockout animals, the C57BL/6 transgenics had higher blood glucose upon glucose challenge, whereas transgenics with a C3H background did not.[20] C57BL/6 mice have the same background that produced the *ob/ob* mice. Moreover, expression of UCP in WAT resulted in substantial fat tissue loss in the C57BL background but had less effect in the $^{Ay}$ background.[19]

The use of transgenics has also served to highlight gender differences in fuel partioning. Several gender differences were observed in the ap2-UCP animals. In the BAT ablation model female mice took longer to achieve similar levels of obesity.[25] But then females achieved much higher levels of obesity and the accompanying parameters. In transgenics that overexpressed PEPCK, only the females had elevated body weights.[30] LPL over expression resulted in decreased body fat in the males only.[11] Thus transgenic animals will provide interesting models in which to further explore differential regulation of nutrient partitioning between the genders.

## Conclusions

In summary, genetic alterations in muscle insulin resistance, BAT levels, glucocorticord receptors, growth hormone, and GLUT4 resulted in excess accumulation of fat mass. Fat mass was diminished by the overexpression of IGF-II, and glycerol 3-phosphate dehydrogenase, and by the elimination of adipose tissue using diphtheria toxin. Diet-induced obesity was attenuated by the overexpression of LPL in muscle and GLUT4 in adipose tissue. Future work with transgenic animals should provide a unique opportunity to study the interaction of the environment, including diet and/or physical activity, on obesity or its prevention. Additionally, transgenic mice will provide relevant models to test obesity-related drugs or potential gene therapies.

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