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METABOLIC LESSONS FROM GENETICALLY LEAN MICE*

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■ **Abstract** Different types of lean mice have been produced by genetic manipulation. Leanness can result from deficiency of stored energy or a lack of adipocytes to store the lipid. Mice lacking functional adipocytes are usually insulin resistant and have fatty livers, and elevated circulating triglyceride levels. Insulin resistance may result from the lack of adipocyte hormones (such as leptin) and increased metabolite (such as triglyceride) levels in nonadipose tissue. Mice with depleted adipocyte triglyceride levels typically are insulin sensitive and have normal or low liver and circulating triglycerides. Mechanisms to produce depleted adipocytes include increased energy expenditure by peripheral tissues, peripheral mechanisms to decrease food intake, and altered central regulation of these processes.

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PHYSIOLOGY OF ENERGY HOMEOSTASIS

The major form of stored energy in mammals is triacylglycerol, commonly referred to as triglyceride. With its energy density of 9 kcal/g, triglyceride is a very efficient way to stockpile energy. Specialized fat-storing cells, adipocytes, are the body's principal storage site and number about half the total cells but the vast majority of the mass of white adipose tissue (WAT). Triglyceride can also be stored in sites other than adipose tissue, such as the liver. A second type of adipose tissue is brown adipose tissue (BAT), whose principal function is to generate heat. BAT heat generation is important in small mammals such as mice, rats, and human infants but is thought to have little or no role in larger mammals such as adult humans. In addition to energy storage and heat generation, adipose tissue also has endocrine functions. Adipose peptide hormones include leptin, adiponectin/ACRP30/AdipoQ, resistin/FIZZ3/ASDF, tumor necrosis factor-α, interleukin 6, plasminogen activator inhibitor 1, and angiotensinogen. In addition, free fatty acids probably have hormone-like actions because some appear to be ligands for the PPAR family of transcription factors.

A lean mouse is the result of a relative excess of energy expenditure over energy intake. Increased energy expenditure can be mediated by peripheral mechanisms such as by directly increasing substrate oxidation in muscle, fat, or other tissues. Decreased energy availability can also occur by peripheral mechanisms, such as by reduced intestinal absorption. The central nervous system (CNS), particularly the hypothalamus, regulates energy intake and expenditure (87). In the past decade our knowledge of the central pathways for regulating energy homeostasis has gone from prehistoric to pre–Industrial Revolution. Mutant mice have led the way, pointing to relevant proteins and neuronal pathways. Thankfully, the molecules and pathways identified in rodents are highly conserved in humans and the murine information is generalizable.

Inputs to the CNS include the hormones leptin and insulin, energy molecules such as glucose and fatty acids, and signals transmitted via peripheral nerves. Leptin is produced by adipocytes in proportion to fat mass and is a major input to the hypothalamus, particularly the arcuate nucleus. Arcuate leptin-responsive neurons include those expressing NPY/AGRP and POMC/CART. CART and αMSH, a POMC cleavage product that stimulates melanocortin-3 and melanocortin-4 receptors, act on paraventricular nucleus neurons, eventually inhibiting food intake. NPY, presumably binding to Y1 and Y5 receptors, and agouti-related protein (AGRP), which antagonizes αMSH binding, stimulate food intake and decrease

energy expenditure. In the lateral hypothalamus, melanin concentrating hormone (MCH) is a neuropeptide that is a potent stimulator of food intake and metabolic efficiency. As discussed below, mutant mice have also identified VGF and muscarinic M3 receptors as important in the food intake/metabolic efficiency regulatory network.

TYPES OF LEAN MICE

Below we discuss about 40 genetically lean mice (we have not included lean mice produced by environmental manipulation) (Table 1). With a data set this size it is possible to derive generalizations about the types of mechanisms causing the leanness. A useful first distinction is between mice lacking normal adipocytes and those in which the adipocytes are triglyceride depleted but otherwise intact and functional. The mice with ablated or malfunctioning adipocytes have insulin resistance (and often actual hyperglycemia); enlarged, fatty livers; and elevated circulating triglyceride levels. In contrast, mice with depleted adipocytes typically are insulin sensitive, have normal sized livers, and have normal or low liver and circulating triglycerides.

A number of different mechanisms can cause triglyceride depletion of adipocytes. In theory, significantly increased metabolism anywhere in the body can cause leanness by a "substrate steal," burning energy that otherwise would be stored in adipose tissue. Some of the best examples of this are transgenic mice with increased muscle oxidation. Adipose tissue can also be a significant contributor to an increased metabolic rate. This can occur in BAT through increased thermogenesis, with secondary triglyceride depletion in WAT. Alternatively, chronic β-adrenergic stimulation of WAT causes more BAT character to the WAT, increasing its metabolic rate. In models of increased peripheral metabolism, food intake is increased, partially compensating for the increased metabolism.

Triglyceride-depleted adipocytes can also result from decreased energy availability originating in peripheral tissues, such as by impaired intestinal absorption. These mice are analogous to underfeeding accomplished by nongenetic means. This group of mice should have a reduced metabolic rate, indicative of CNS detection and an attempt at compensation for the reduced energy intake.

Another class of mechanisms for producing lean mice with triglyceride-depleted adipocytes is via altered CNS regulation. This group is heterogeneous. It might be subdivided on the basis of what the apparent primary effect is, such as decreased food intake (with compensatory decreased energy expenditure), increased energy expenditure (with compensatory increased food intake), or both decreased food intake and increased energy expenditure. A complementary way to subclassify this group is by the changes in hypothalamic neuropeptide levels, looking for changes from the usual patterns. Matching the neuronal and physiologic changes will push our understanding to new levels.

There are also genetic models that do not fit the patterns. These can be the most interesting mice, providing discrepancies to be deciphered for mechanistic

TABLE 1 Numbers generally are from young adult mice and should be considered approximations for comparison between mice, due to such differences as genetic background, age, sex, and the laboratory doing the work. For insulin, glucose, and triglyceride levels, numbers are listed only when significantly elevated

Gene description	Type of mutation, site of expression	Tissue affected	WAT	BAT	Liver size	Liver triglyceride	Insulin sensitivity	Insulin (ng/ml)	Glucose (mg/dl)	Triglyceride (mg/dl)	Leptin (ng/ml)	Food intake	Physical activity	Metabolic rate	Reference
1. Peripherally driven increased energy expenditure via muscle															
myosin light chain 2 driving UCP1	tg, muscle	muscle	60%				↑	↓	↓	↓		↑	N	↑	(60)
α-skeletal actin driving UCP3	tg, muscle	muscle	50%					↓	↓	N	N	↑	N	↑	(14)
creatine kinase driving LPL	tg, muscle	muscle	↓				var	N, ↑	N, ↑	↓		N, ↑			(43, 59)
2. Peripherally driven increased energy expenditure via adipose tissue															
aP2 driving β1 adrenergic receptor	tg, adipose	fat	75%	N											(93)
aP2 driving UCP1	tg, adipose	fat	var	N	N										(51)
RIIβ subunit of protein kinase A	ko	fat	50%	N				N	N	N	↓	↑			(17, 79)
translation inhibitor 4E-BP1	ko	fat	40%	N				N	↓	N	↓			↑	(97)
aP2 driving FOXC2 transcription factor	tg, adipose	fat	20%	4x↑	N	N	↑	↓	↓	↓					(12)
3. Increased energy expenditure via multiple or unknown tissues															
overexpress glycerol 3-P dehydrogenase	tg, wide	multiple	25%	2x↑											(53, 54)
protein tyrosine phosphatase-1B	ko	?liver/muscle	30%	N			↑	↓	↓	↓	↓	N, ↑		↑	(23, 48)

acetyl-CoA carboxylase 2	ko	?muscle	50%			↓			↓	↑	↓	↑		↑	(1)
complement C3	ko	unknown	50%	N	N		↑	N, ↓	N, ↓	N	↓	↑			(72)
G protein as subunit	+/p- ko, paternal allele	?CNS	50%	smaller, active			↑	↓	↓	↓	↓	N, ↑	↑	↑	(110–112)
acyl CoA:diacylglycerol transferase	ko	?fat	50%	N				N	N	N	↓	N			(91)
4. CNS-mediated lean mice															
serum amyloid P driving leptin	tg, liver	CNS	none visible	N, then disappears	0.76x	↓	↑	↓	N	↓	↑ (tg)	↓			(67, 74)
aP2 driving leptin	tg, adipose	CNS	10%	30%, less lipid	1.4x↑		↑	↓	↓	↓	↑ (tg)	↓			(80)
attractin (mg-3J allele)	natural ko	CNS	70%									N, ↑	↑	↑	(22, 32)
melanin concentrating hormone	ko	CNS	55%					N	N		↓	↓		↑	(88)
neuropeptide Y	ko	CNS													(24, 25, 38)
muscarinic M3 receptor	ko	CNS	50%				↑	↓	N	↓	↓	↓	N	N	(68, 108)
VGF	ko	CNS	30%					↓	↓		↓	↑	↑	↑	(34, 85)
5. Peripherally decreased energy availability															
CMV/actin driving pancreatic polypeptide	tg, wide	unknown	↓					N	N		↓	↓	N	N	(98)
apoB knockout, with liver apoB	ko with tg liver	intestine	15%												(44, 109)
VLDL receptor	ko	muscle/fat	50%	N	N			N	N	N		N			(26)

(*Continued*)

TABLE 1 (*Continued*)

Gene description	Type of mutation, site of expression	Tissue affected	WAT	BAT	Liver size	Liver triglyceride	Insulin sensitivity	Insulin (ng/ml)	Glucose (mg/dl)	Triglyceride (mg/dl)	Leptin (ng/ml)	Food intake	Physical activity	Metabolic rate	Reference
6. Special cases															
C/EBPβ	ko	multiple	50%	less lipid			↑	N	N	N					(4, 62, 94, 99)
C/EBPδ	ko	multiple	70%	less lipid											(94)
C/EBPβ C/EBPδ double ko	double ko	multiple	30%	less lipid											(94)
HMGI-C	ko, 4 times	?fat	20%									N, ↑			(2, 5, 7 107, 113)
GLUT4	ko	fat/muscle	15%				↓	↑ var	↓ var						(45, 96)
metallothionin driving TGFα	tg, wide	?fat/multiple	50%											N	(64)
keratin driving TNFα	tg, skin	?fat/multiple	none visible												(13)
7. Abnormal adipocyte biochemistry															
perilipin	ko	fat	30%	less lipid	N	N		1.2	↑	N	↑	N			(66, 95)
8. WAT ablation															
C/EBPα knockout, with liver C/EBPα	ko with tg liver	fat/multiple	var	N	2x↑	↑		5	N	1100	↓				(61)
aP2 driving dominant negative A-ZIP/F	tg, adipose	fat	1%	N, fast involution	2.1x↑	↑	↓	100	600	800	↓	↑	N		(27–29, 71)
aP2 driving constitutively active SREBP-1c	tg, adipose	fat	35%	4x↑	1.8x ↑	↑	↓	20	300	110	↓				(89, 90)
aP2 driving modified diphtheria toxin	tg, adipose	fat	10% @ 10 mo.	reduced @ 10 mo.	3.5x ↑	↑	↓	12	250	120	↓	↑			(9, 84)
PEPCK driving TGFβ1	tg, wide	fat/multiple	10%	1/3 of N weight	1.2x ↑	↑				170					(15)
lipin-1 (fld and fld2J alleles)	natural mutations	fat	20%	reduced in adult	1.2x ↑	↑		4	N		↓				(77, 78, 82)

Key: N, normal; tg, transgene; ko, knockout; var, variable.

insight. Finally, a reduced fat mass can also be a "nonspecific" manifestation of a sick animal. This is not to dismiss the effects of illness on energy hemostasis, but in severely ill mice it is difficult to sort out the specific effects on energy homeostasis (e.g., role of cytokines) from indirect effects (e.g., it hurts to move, so the mouse does not eat). With a possible exception (13), we have not included severely ill mice in this review, unless they provide mechanistic insight.

EXPERIMENTAL ASSESSMENT OF ALTERED ADIPOSITY AND ITS CAUSES

Measuring Adiposity

Before examining particular mouse models we review a few points about the measurement and evaluation of adiposity and the phenotyping of mice. The ideal method would allow repeated measurement over time of the same mouse, while providing information about regional fat distribution. Body weight, while an imperfect measurement, is easy to measure accurately and to follow over time in a live mouse. More specific is the body mass index (BMI), weight divided by length squared. In the mouse the nose to anus length is used. Whereas the BMI corrects for differences in body size, it usually requires an anesthetized mouse and the same observer for accurate, reproducible data. A more sensitive and specific measurement of adiposity is fat pad weight. Other techniques used to measure adiposity include carcass triglyceride content, dual energy X-ray absorptiometry, nuclear magnetic resonance spectroscopy, magnetic resonance imaging, and computerized tomography.

Scaling Measurements to Body Weight

Leanness and obesity result from an imbalance between energy intake and expenditure. Thus, the determinants of fat mass are net energy intake and energy expenditure, with the efficiency of energy expenditure and partitioning of energy to or from fat being important secondary determinants. Small but chronic differences in energy balance, if sustained, have a profound effect on fat stores. Experimentally, food intake is the commonly used measure of net energy intake (the calories lost in urine/feces are often not quantitated, and in most cases this will not significantly affect the conclusions). Energy expenditure in mice is usually measured as oxygen consumption using indirect calorimetry (69).

Energy intake and expenditure vary with body size; thus, it is important to normalize these measurements. Observations by Kleiber revealed an empiric fit to the 3/4 power of body weight (49, 50), and a first principles explanation for such "allometric" scaling was proposed. Briefly, the 3/4 power results from the constraints of maximally efficient transport (of nutrients and waste) within the body, which presumably has been selected for during evolution (6, 103, 104).

WAT can vary widely in amount (from <2% of body weight in lipoatrophic mice to ~80% in mice lacking leptin). Because WAT consists chiefly of stored, inert triglyceride, which does not contribute to metabolic activity, normalization to (body weight)$^{3/4}$ is not appropriate for comparing animals with different percentages of body fat. Ideally, the most accurate normalization is to (lean body mass)$^{3/4}$. For groups of animals with the same fat mass and body weight, normalization is not required but tends to reduce the error.

Importance of Ambient Temperature in Smaller Mammals

Thermoregulation is profoundly different in homeotherms (warm-blooded animals) with small bodies and those with large bodies. Heat loss is rapid in small animals, owing to their high surface area:volume ratio, and consequently they normally burn calories to keep warm (called facultative thermogenesis). Small mammals also compensate for heat loss by behavioral adaptation (nesting, huddling). In contrast, maintaining body temperature is relatively easy for adult humans, who rely predominantly on behavioral adaptation (clothes, creating a warm environment) and not thermogenesis for keeping warm.

The thermoneutral zone, defined as the range of ambient temperatures at which the metabolic rate is at a minimum (30), is broad for adult humans, extending from ~24° to >37°C (86, 105) (Figure 1). Indeed, decreasing the ambient temperature from 28° to 22°C increases the metabolic rate by only 7% (19). The thermoneutral zone in mice is ~30–34°C (30, 37), and mice show huge increases in metabolic rate at environmental temperatures below thermoneutrality [22°C causes an increase of 80% and 4°C, an increase of 320% (35)]. The ability of mice to profoundly increase metabolic rate can have major effects. For example, the difference of a few degrees in ambient temperature measurably changes food intake and metabolic rate. Experimentally this can be addressed by measurement of food intake and metabolic rate at thermoneutrality.

From the facts discussed above, it follows that body temperature is not a surrogate measure of metabolic rate. Figure 1 illustrates this lack of correlation between metabolic rate and body temperature.

Effects of Sex, Age, Diet, and Background Genotype

There are differences, sometimes profound, between male and female mice in leanness/obesity and diabetes phenotypes. Leanness/obesity can be more significant in one sex (75). Insulin resistance/diabetes phenotypes are often worse in male mice (58).

As mice age they generally become more obese. There are transgenic mice that exaggerate this process (80) but also mice that do the opposite (9). The probability of diabetes generally increases with age, but in some models hyperglycemia improves with age (16, 58).

Diet has a major effect on leanness and insulin resistance. Typical rodent chow has 12% of calories as fat. Increasing the fat content increases the probability

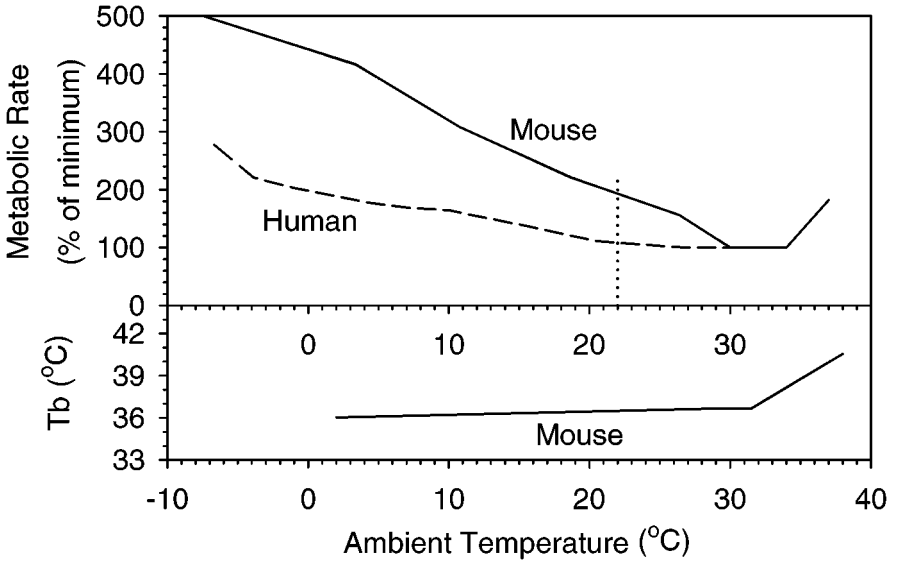

Figure 1 Metabolic rate and body temperature (Tb) as a function of ambient temperature. The dotted line marks room temperature, showing the small increase in human and the large increase in mouse metabolic rate, compared with thermoneutrality. Data are from the following sources: human metabolic rate (*dashed line*) (40), mouse metabolic rate (*solid line*) (35, 37), and mouse body temperature (39).

of obesity and insulin resistance. This is one of the common ways to check for resistance to obesity in mouse models. The type of dietary carbohydrate is also important. For example, high fructose diets are more diabetogenic.

Another important influence is background genotype. Huge effects have been reported on body fat content and response to a high fat diet (102). Different inbred strains can show a 130-fold variation in WAT uncoupling protein-1 (UCP1) mRNA levels (31). Background genotype can mean the difference between normal glucose levels and diabetes. Thus, mice should be on a pure genetic background or compared with littermate controls.

SPECIFIC EXAMPLES OF GENETICALLY LEAN MICE

Peripherally Driven Increased Energy Expenditure via Muscle

Two groups have produced lean transgenic mice with increased energy expenditure in muscle owing to uncoupling proteins. Li et al. expressed UCP1 in skeletal muscle (60). UCP1 is normally found only in BAT and uncouples substrate oxidation from ADP phosphorylation by causing the mitochondrial inner membrane to become

leaky to protons. Clapham et al. overexpressed a related protein, UCP3, in muscle (14), although the increased leak was apparently not due to normal functioning of the UCP3 protein (10). In both models body weight was reduced, as was the fraction of body weight that is fat. Whole body and isolated muscle metabolic rates increased. These data suggest that the UCP transgenes increased muscle proton leak, causing a secondary increase in substrate oxidation in order to maintain ATP levels. The mice increased their food intake to replenish their energy stores, but this did not completely compensate, leading to thinner, leaner mice. Both models show increased whole body insulin sensitivity.

These mice are the clearest examples of transgenic mice that become lean by a primary increase in energy expenditure. Like nongenetic models of leanness, such as caloric restriction or increased exercise, these mice show increased insulin sensitivity. It is interesting to note that the UCP1-overexpressing mice had a small but significant increase in muscle triglyceride (60), similar to endurance-trained humans (76). These provocative experiments raise a number of questions. Does increased uncoupling in muscle have antidiabetic effects above and beyond those caused by decreased fat mass? Would increased uncoupling in liver have the same effect? What about increased uncoupling in a tissue that is not insulin responsive? Are there untoward effects caused by chronically elevated uncoupling?

Mice expressing lipoprotein lipase (LPL) in muscle become lean in proportion to the degree of overexpression (59). This occurs despite normal (increased for body size) food intake, indicating that the increased delivery of fatty acid to muscle must stimulate metabolism in some way. Lower levels of muscle LPL expression cause resistance to dietary or genetic (*ob/ob*) obesity but not leanness in the absence of such a stress (43, 101). However, these low levels of muscle LPL do cause isolated muscle insulin resistance (46). A likely explanation is that increased intracellular fatty acids or their metabolites contribute to insulin resistance. As might be predicted, LPL deletion is a lethal phenotype, with the mice being unable to deposit triglyceride into their WAT before they die (100).

Peripherally Driven Increased Energy Expenditure via Adipose Tissue

The major stimulus for heat production by BAT and lipolysis in WAT is β-adrenergic stimulation via the sympathetic nervous system. In BAT, this signals via protein kinase A and PPARγ coactivator-1 (PGC1) to increase Ucp1 levels and activate uncoupling. The mouse models in this section have in common activation of this pathway, albeit by different methods and at different sites.

Mice overexpressing the β1-adrenergic receptor in adipose tissue had smaller adipose tissue depots, showed an increased lipolytic response to β-adrenergic stimulation, and had brown fat cells appearing in traditionally white adipose depots (93). These changes mimic those seen in mice chronically cold-challenged or treated with a β3-adrenergic agonist. Because lean mice and mice treated with

a β3 agonist are more insulin sensitive, the β1 overexpressors may be insulin sensitive, although this was not reported.

Transgenic mice expressing Ucp1 in adipose tissue were resistant to obesity caused either by ectopic agouti expression (A^y) or a high fat diet (51, 52). Interestingly, the change in fat pad weights was gender dimorphic and region specific (reduced femoral and mesenteric WAT, no change in renal WAT, and an increase in male gonadal WAT) (51). The presumed mechanism in these mice is the excess energy expenditure by the Ucp1 transgene in white and brown adipose tissue.

The lean phenotype resulting from disruption of the RIIβ subunit of protein kinase A was quite unexpected (17). This gene is most abundantly expressed in adipose tissue and brain. In compensation for RIIβ ablation, the mouse upregulates the RIα isoform. Protein kinase A holoenzyme containing RIα is activated at lower cAMP levels, leading to a higher adipose tissue protein kinase A activity in response to β-adrenergic stimulation (17, 79). The result is a lean phenotype owing to increased WAT lipolysis and BAT thermogenesis. In this mouse one must remember that RIIβ is also expressed in the brain, and ablation has behavioral effects (8).

Translation inhibitor 4E-BP1 is expressed at high levels in multiple tissues, including WAT, BAT, and pancreatic islets. Phosphorylation of 4E-BP1 disinhibits translation and occurs in response to treatment of adipocytes with insulin. Mice lacking translation inhibitor 4E-BP1 (*Eif4bp1−/−*) were lean and hypoglycemic (97). The *Eif4bp1−/−* WAT had BAT-like characteristics presumably owing to increased PGC-1 translation, leading to increased Ucp1, BAT histology, and increased energy expenditure.

In adult mice the Foxc2 is a transcription factor that is expressed selectively in adipose tissue. *Foxc2−/−* mice fail to live past the neonatal period (41, 106); the WAT phenotype of *Foxc2+/−* mice has not been reported. Transgenic FOXC2 overexpression in adipose tissue produced lean, insulin-sensitive mice with BAT hypertrophy (12). The WAT in these mice expressed Ucp1, consistent with a partially BAT-like histology. These results suggest that transgenic adipose overexpression of FOXC2 causes a lean phenotype through increased adipose tissue energy expenditure. The role of *Foxc2* in the regulation of normal adipose tissue remains to be examined.

Increased Energy Expenditure via Multiple or Unknown Tissues

Mice overexpressing cytoplasmic glycerol 3-phosphate dehydrogenase are lean (54). When bred to *db*/*db* mice, the transgene reduced the body weight to wild-type levels (53). The likely explanation is that these mice have increased basal metabolism and heat production. Specifically, cytoplasmic and mitochondrial glycerol 3-phosphate dehydrogenases together constitute the glycerol phosphate shuttle, an energetically inefficient (and thus heat-producing) way to move reducing equivalents from the cytoplasm into the mitochondrion [reviewed in (20)]. Consistent with this hypothesis is the observation that the BAT, although capable of

activating in response to cold, was inactive, suggesting that it was dormant, compensating for increased heat production elsewhere (54). Insulin sensitivity has not been measured in these mice, but they are predicted to be more sensitive.

Mice lacking acetyl-CoA carboxylase 2 (*Acc2−/−*) are lean (1). The reduction in muscle and heart acetyl-CoA carboxylase activity causes a decrease in malonyl CoA, activating carnitine palmitoyl transferase-1 and thus increasing fatty acid oxidation. The increased muscle fatty acid oxidation and metabolic rate result in less triglyceride in adipose tissue and liver and an increase in food intake in an attempt to compensate (1). Alternatively, one cannot rule out a CNS effect from the loss of neuronal *Acc2*.

Ablation of the protein tyrosine phosphatase-1B gene results in increased or prolonged insulin receptor phosphorylation in liver and muscle but not in adipose tissue (23). These mice have lower blood glucose, insulin, and triglyceride levels and are more insulin sensitive in muscle but not adipose tissue (23, 48). The mice have normal or increased food intake despite being obesity resistant; this is explained by an increased metabolic rate (48). The knockout phenotype may be explained by augmented insulin action in liver, muscle, and/or CNS causing increased substrate oxidation, making less available for storage in WAT. Another possible mechanism is increased phosphorylation of insulin-independent pathways.

Mice lacking complement C3 are immune deficient and lean (72). Because they are lean despite increased food intake, the mice must be hypermetabolic, but the involved tissues are not known. A possible mechanism for the phenotype is the deficiency of C3adesArg, also known as acylation-stimulating protein, which is proposed to regulate fatty acid uptake in adipocytes (92).

A mouse with a lean phenotype was observed with paternal transmission (+/p−) of the $G_s\alpha$ knockout allele (111). $G_s\alpha$ is the stimulatory α subunit, via which G-protein-coupled receptors increase cAMP. The *Gnas* gene is imprinted in a tissue-specific manner, meaning that in certain tissues only one parental allele is expressed (112). Uncovering the primary event in the +/p− mice responsible for their leanness is difficult because there are hundreds of G-protein-coupled receptors. A working model is that cAMP signaling from certain G-protein-coupled receptors is impaired. This causes increased sympathetic tone to WAT and BAT, an increased resting metabolic rate, and increased physical activity (111). Food intake is also increased, possibly secondary to the increased energy expenditure. Insulin sensitivity in the +/p− mice is increased (110).

An intriguing lean, insulin sensitive mouse resulted from ablation of the acyl-CoA:diacylglycerol transferase (DGAT) gene, which encodes the enzyme that converts diacylglycerol to triglyceride. Initially, it was surprising that *Dgat1* knockout mice have normal circulating triglyceride levels (91), but further investigations identified additional DGAT genes (11). In the *Dgat1* knockout mice, fat pad weights were reduced by 60–80%, and carcass triglycerides were reduced by 33%. Glucose tolerance results suggested increased insulin sensitivity. The lean phenotype appeared to result from increased physical activity and metabolic rate and not decreased food intake. It is not known how ablation of the *Dgat1* gene causes the lean phenotype, not even which tissue is the primary site (adipose, brain, other).

It is tempting to speculate that *Dgat1* ablation causes abnormal CNS sensing of energy stores, for example by analogy to the lean phenotype caused by inhibition of fatty acid synthase (63).

Central Nervous System–Mediated Lean Mice

Some lean transgenic mice have an altered energy homeostasis causing them to act physiologically as if they have ample or excessive energy stores when this is not the case. The hallmark of CNS regulation is that multiple mechanisms are involved, such as decreasing food intake and increasing physical activity and other forms of energy expenditure. However, some alterations of CNS regulation affect only some of the inputs to energy homeostasis.

One way to affect all of the CNS regulation is to increase the circulating leptin levels. Most investigators have done this with injections or short-term continuous infusions. Transgenic expression allows longer treatments and higher doses. Mice secreting leptin from adipose tissue (42, 80) and liver (67, 74) have been produced.

The transgenic mice with the highest leptin levels (~10 times higher than littermates and ~100 times higher than expected for the degree of fat mass) were lean with no visible WAT and decreased food intake (74). The mice had decreased circulating and hepatic triglyceride levels and increased insulin sensitivity. Based on known effects of leptin, it is likely that these mice were also less metabolically efficient and expended more energy. Interestingly, one of the leptin-overexpressing models with a low adipose mass at 6–9 weeks of age had normal adipose stores at 33–36 weeks, despite continued elevation of leptin levels (80).

Downstream of leptin are the proopiomelanocortin (POMC) neurons in the arcuate nucleus of the hypothalamus. These POMC neurons secrete α-melanocyte stimulating hormone (αMSH), an agonist of melanocortin-3 and melanocortin-4 receptors. The melanocortin pathway is anorexigenic; thus, activators of this pathway should produce lean mice. To date, no transgenic mice that directly activate this pathway have been reported. αMSH action is inhibited by agouti-related protein (Agrp); an Agrp knockout is also predicted to be lean (21).

The *mahogany* mutations were identified as lessening the effects of ectopic *agouti* expression in A^y/a mice. It is now known that the *mahogany* mutations are in the attractin gene and range from null to hypofunctional alleles (33, 73). The attractin protein binds and inhibits the action of agouti, but not of Agrp, thus this mechanism for attractin's effect on energy homeostasis is restricted to models that ectopically express agouti protein (36). However, attractin has also been shown to reduce adiposity and increase physical activity in the absence of ectopic agouti expression (22, 32). These effects of attractin mutations are likely due to other, less specific CNS effects, because attractin mutations also cause hypomyelination and neuronal degeneration (55). The *mahoganoid* (*md*) mutation also suppresses A^y obesity (70). However, the molecular basis for the *md* mutation and whether it acts specifically on AGRP are unknown.

Melanin concentrating hormone (MCH) is a neuropeptide acting in the lateral hypothalamus that increases food consumption (81). It is also downstream of

leptin. Knockout mice lacking MCH are lean owing to both decreased food intake and increased metabolic rate (88). These occurred despite reduced circulating leptin levels and arcuate nucleus POMC mRNA levels, confirming that MCH acts downstream of leptin and αMSH signaling. Glucose and insulin levels were slightly (but not statistically significantly) reduced in the MCH knockout mice, suggesting that these mice tend towards insulin sensitivity.

NPY was one of the first orexigenic neuropeptides identified, and central delivery of NPY remains one of the most potent orexigenic stimuli known. Thus, when NPY−/− mice were generated it was surprising that they were of normal weight and food intake, including hyperphagia following food deprivation (24). Some subtle changes in energy homeostasis in the NPY−/− mice were identified, including augmented decrease in food intake following treatment with leptin (24). When mice doubly ablated for NPY and leptin were studied they showed less obesity, less food intake, and greater energy expenditure than singly leptin deficient mice (25). Possible explanations for the modest phenotype of the NPY−/− mice include redundancy in the energy homeostasis pathways and plasticity during development in the absence of NPY.

Hypothalamic neurons have both Y1 and Y5 NPY receptors, and mice lacking either of these receptors have been produced. Although both mutants show slightly decreased food intake in response to intracerebro-ventricular administration of NPY agonists, both also became obese (65, 75). These data suggest that NPY, acting via the Y1 and Y5 receptors (and possibly others), does affect food intake. However, compensatory changes in energy homeostasis (either directly via Y1 and Y5 receptors or indirectly) also occur, leading to increased adiposity.

A lean phenotype was observed in mice lacking the M3 muscarinic receptor (68, 108). The mechanism appears to decrease food intake owing to altered hypothalamic regulation (108). Hypothalamic AGRP mRNA levels were increased, while POMC (αMSH) mRNA levels were reduced, consistent with the low body energy stores. In contrast, hypothalamic MCH mRNA levels were reduced, which is not seen in lean mice. Central infusion of $AGRP_{83\text{-}132}$, a melanocortin antagonist, did not increase food intake, but MCH did. These data suggest an M3R effect downstream of αMSH/AGRP and upstream of MCH.

VGF is a secreted neuropeptide whose hypothalamic mRNA levels are induced by fasting. VGF-ablated mice have a remarkably severe phenotype consisting of leanness with increased activity and metabolic rate (34). Hypothalamic mRNA levels of NPY and AGRP were increased and POMC was decreased, consistent with a fasting situation and suggesting that VGF ablation affects pathways downstream of these neurotransmitters.

Peripherally Decreased Energy Availability

A lean, insulin-sensitive phenotype should result from genetic manipulations to decrease energy intake, analogous to a hypocaloric diet. Decreased food intake could result from decreased appetite, as part of a changed CNS energy store set point (discussed above). Genetic manipulation of gastrointestinal communication

to the brain might include increasing oleylethanolamide signaling (83). Mice overexpressing pancreatic polypeptide weigh less and have less food intake and decreased gastric emptying (98), and thus are possibly a genetic model for decreased food intake. Decreased intake could also result from poor gastrointestinal absorption. One would predict that (partial) loss of pancreatic lipase would result in such a mouse, mimicking the action of orlistat, a lipase inhibitor used clinically (3). Deficiency of intestinal apoB prevents triglyceride uptake by lack of chylomicron formation, leading to profound leanness (44, 109). It is interesting that mice lacking the VLDL receptor have 50% reduced fat mass, presumably owing to decreased triglyceride transport to muscle and adipose tissue (26). No differences in glucose or insulin levels were noted.

Special Cases: Confusing or Insufficient Information

Both the C/EBPβ and C/EBPδ transcription factors, while widely expressed, have been implicated in adipocyte development. Many C/EBP$\beta-/-$ mice die perinatally and the rest are lean (94), with low liver glycogen levels, fasting hypoglycemia, impaired hepatic glucose production, impaired adipocyte lipolysis, and increased muscle insulin sensitivity (4, 62, 99). Thus, the phenotype seems to be determined by the paucity of available energy in the mice. Mice lacking C/EBPβ selectively in adipose tissue will be required to determine the relative contribution of liver, adipose, and other tissues in causing the phenotype.

C/EBP$\delta-/-$ mice are lean, but the WAT and metabolic phenotypes have not been studied in detail (94). C/EBP$\beta-/-$C/EBP$\delta-/-$ double knockout mice are leaner than either the C/EBP$\beta-/-$ or C/EBP$\delta-/-$ mice and many die early (94). The uncertainty noted for the individual C/EBP$\beta-/-$ or C/EBP$\delta-/-$ mice also applies to the C/EBP$\beta-/-$C/EBP$\delta-/-$ mice.

HMGIC is an "architectural" transcription factor, inactivation of which causes the *pygmy* phenotype (5, 7, 107, 113). Pygmy mice are proportionately small, with disproportionately greater fat loss. HMGIC is expressed in proliferating mesenchymal cells and not in most adult tissues, with adipose tissue undergoing expansion being one exception. Thus, the *Hmgic−/−*mice are proposed to be resistant to adipose tissue cell division but not hypertrophy (2).

Ablation of GLUT4, the insulin-sensitive glucose transporter, produced very lean mice, presumably owing to the diminished ability of the WAT to take up glucose (45). The mice were resistant to insulin's hypoglycemic effect, which was remedied by transgenic replacement of muscle GLUT4 (96).

Mice overexpressing TGFα have a complex phenotype that includes reduced fat depots with normal food intake (64). When crossed with *ob/ob* mice, the *ob/ob* obesity was only slightly reduced. TGFα repressed adipose differentiation in cell culture, but the mechanism for its induction of lean mice is unknown.

Abnormal Adipocyte Biochemistry

Perilipin is a protein that coats the adipocyte lipid droplets and modulates hormone-sensitive lipase activity. Perilipin knockout mice have increased basal lipolysis

(66, 95). Anatomically they have a reduced adipose mass and an increased muscle mass. Metabolic rate per unit body weight is increased, owing, at least in part, to the increased muscle mass. It is not clear how increased lipolysis would cause increased muscle size; possibly an augmented fatty acid supply causes growth either via a nutritive or hormonal mechanism. Another potential contribution to an increased metabolic rate could be a futile cycle of lipolysis and re-esterification. In virtually all lean mice the circulating leptin levels are reduced in proportion to the degree of fat loss (and thus leptin usually is not particularly useful for characterizing lean mice). The peri−/− mouse also produces leptin in proportion to fat mass, but at an approximately fivefold greater level. Maybe the peri−/− mouse holds the answer to how increased adipocyte triglyceride causes increased leptin transcription.

White Adipose Tissue Ablation

Multiple mouse models involving ablation of WAT have been reported. As noted above, characteristics of all WAT-ablated mice include insulin resistance, fatty liver, and increased circulating triglyceride levels.

C/EBPα is a transcription factor important for the expression of many adipocyte genes. *c/ebp*α−/− mice die within hours of birth with hypoglycemia, owing to defective gluconeogenesis (18). The adipose tissue in the *c/ebp*α−/− mice failed to accumulate lipid, but this was difficult to interpret owing to the other affected tissues. Thus, the role of adipocyte C/EBPα was investigated using transgenic hepatic C/EBPα expression in the *c/ebp*α−/− mice (61). The resulting mice had near-normal BAT and mammary WAT but absence of subcutaneous, perirenal, and epididymal WAT. Furthermore, the mice had high triglyceride levels and fatty liver, along with high insulin levels, suggesting insulin resistance. Despite the effects of C/EBPα deficiency in other tissues, these data suggest that the lack of adipose C/EBPα causes the observed phenotype and raises the possibility that C/EBPα is not important for BAT development and function.

Three groups used the aP2/422 adipose fatty acid binding protein promoter/enhancer to drive transgene expression selectively in adipose tissue. The first group to make WAT-ablated transgenic mice used expression of a modified diphtheria toxin to produce the aP2-DT-A mice (84). They observed different phenotypes. Offspring of certain mosaic founders died soon after birth with chylous ascites, presumably owing to transgene expression levels that were not compatible with life. A strain expressing much less DT-A transgene initially appeared normal. However, at >6 months of age these mice showed decreased fat mass and were insulin resistant (9). This strain was also resistant to hypothalamic obesity produced by monosodium glutamate injection into newborns.

The most severe viable adipose deficiency model is the A-ZIP/F-1 transgenic mouse. The transgene is a dominant negative protein that heterodimerizes with and inactivates members of the C/EBP and JUN families of B-ZIP transcription factors (71). Features of the model include virtually no visible WAT at any time during development but near normal BAT at birth, which undergoes accelerated,

premature involution, becoming reduced in amount and inactive. The livers of the A-ZIP/F-1 mice are enlarged and engorged with lipid, but show little hepatitis. The mice have elevated serum free fatty acids and triglycerides and reduced leptin levels. The A-ZIP/F-1 mice are severely diabetic, with remarkably elevated (50- to 400-fold) insulin levels (71). Both the muscle and liver are severely insulin resistant, even before the onset of overt hyperglycemia at 4 weeks of age (47). The β-cell number of the pancreatic islets is increased, particularly in older mice.

The third WAT-ablated transgenic mouse expresses a constitutively active form of the SREBP-1c transcription factor (90). The WAT-deficient phenotype of the aP2-nSREBP-1c mice was a surprise because SREBP-1c is thought to stimulate lipogenesis. The phenotype of the aP2-nSREBP-1c mice is slightly less severe than that of the A-ZIP/F-1 mice, with a reduction in WAT mass of $\sim$70%. They also have hepatomegaly and hepatic steatosis without hepatitis and elevated blood triglyceride and low leptin levels. aP2-nSREBP-1c mice have insulin resistance with hyperglycemia and hyperinsulinemia (90). Sufficient WAT was present to allow characterization of its gene expression profile. The WAT showed increased expression of Pref-1, a marker of preadipocytes, and decreased expression of genes characteristic of fully differentiated adipocytes (PPARγ, C/EBPα, adipsin, aP2, and leptin).

The BAT of the aP2-nSREBP-1c mice was larger, whiter, and contained more lipid droplets than did control BAT. The BAT showed increased Pref-1 expression and decreased PPARγ, C/EBPα, adipsin, aP2, and UCP1 expression. The enlarged, immature BAT phenotype of the aP2-nSREBP-1c mice is different from the premature involution phenotype of the A-ZIP/F-1 mice. These differences presumably reflect the underlying transgene effects.

The fatty liver dystrophy (*fld/fld*, now formally, *lpin1^{fld}/lpin1^{fld}*) mouse is a spontaneously arising model of WAT deficiency. The phenotype of the *fld/fld* mouse includes fatty liver and hypertriglyceridemia, which both resolve by weaning, and a peripheral neuropathy that does not (56, 57). The *fld/fld* mice have 50–90% reductions in WAT and BAT mass (in young and old mice) and mild insulin resistance (a two- to fourfold increase in insulin levels) without hyperglycemia (82). Recently, causative mutations were identified in the *Lpin1* gene encoding lipin (78). Lipin is expressed at highest levels in differentiated adipose tissue, testis, and muscle and at lower levels in other tissues, including liver. Lipin is an 891–amino acid nuclear protein of unknown function. The insulin resistance of the *fld/fld* mouse may result from the WAT deficiency, but this has not been confirmed experimentally. The causal relationships between the various abnormalities in the *fld/fld* mouse are unclear.

CONCLUSIONS

By classifying the reported lean mouse models, some general principles have become clear. Most obvious is the distinction between adipose tissue ablation and low adipose triglyceride stores. All of the mice discussed in this review were

reported in the past 10 years. Whereas an infinite percentage increase in the number of lean mice cannot occur in the next decade, the future still holds much promise for the study of genetically lean mice. They should continue to teach us the peripheral and CNS pathways, mechanisms, and physiology of energy homeostasis.

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