

Tissue-specific Ablation of the GLUT4 Glucose Transporter or the Insulin Receptor Challenges Assumptions about Insulin Action and Glucose Homeostasis*[§]

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The prevalence of type 2 diabetes mellitus is growing worldwide. Most forms of type 2 diabetes are polygenic with complex inheritance patterns strongly influenced by environmental factors. The specific gene defects are unknown, but they affect both insulin action and insulin secretion. Glucose homeostasis is maintained by the fine orchestration of insulin secretion and insulin action to promote glucose transport into muscle and adipocytes and to inhibit hepatic glucose output. Resistance to these effects of insulin is a classic pathogenic feature of obesity and type 2 diabetes. Insulin action on lipid metabolism also has important effects on glucose homeostasis. Recent studies using tissue-specific gene targeting of the GLUT4 glucose transporter or the insulin receptor in mice reveal intercommunication among insulin target tissues which can modify the impact of genetic defects in individual tissues. These studies provide new concepts regarding the importance of adipose tissue *versus* muscle in whole-body insulin sensitivity and the role of proximal *versus* distal components of the insulin action cascade in the pathogenesis of obesity and type 2 diabetes.

The insulin receptor (IR)¹ is present in virtually all mammalian cells, and insulin binding results in activation of several phosphorylation-dephosphorylation cascades (1). The phosphoinositide 3-kinase cascade is necessary, albeit insufficient, for stimulation of glucose transport, and the mitogen-activated protein kinase cascade underlies the mitogenic effects of insulin. These insulin signaling cascades have pleiotropic metabolic and growth-promoting effects (see Fig. 1 in Supplemental Material).

Insulin stimulation of glucose transport in muscle and adipose cells is essential for maintenance of glucose homeostasis. This is mediated by sodium-independent, facilitated-diffusion glucose transporters (GLUTs). In tissues with insulin-sensitive glucose transport (*i.e.* skeletal and cardiac muscle and adipose cells), GLUT4 is the predominant glucose transporter and GLUT1 plays a minor role. The large stimulatory effect of insulin in these tissues

results from the translocation of GLUT4-containing vesicles from intracellular storage sites to the plasma membrane where they dock and fuse with the membrane (2, 3), markedly augmenting glucose transport into the cell.

GLUT4 is present primarily in white and brown adipocytes, skeletal muscle, and cardiac muscle, with expression in discrete areas of other tissues (*e.g.* brain and kidney) that are not traditionally thought to play a major role in glucose homeostasis (4). In insulin-resistant states including obesity and type 2 diabetes, GLUT4 expression is reduced in adipocytes but not in skeletal muscle (4, 5). This down-regulation in adipocytes was not thought to be important because skeletal muscle accounts for up to 85% of glucose disposal following a glucose infusion (6) and at least 50% following glucose ingestion (7), whereas adipose tissue accounts for much less. Glucose transport is the rate-controlling step in skeletal muscle glucose metabolism in normal and type 2 diabetic subjects (8). Impaired glucose uptake in skeletal muscle is present even in non-diabetic relatives of type 2 diabetic subjects and is a risk factor for developing diabetes (9, 10). Defects in GLUT4 trafficking or function in skeletal muscle are thought to be most important in the development of insulin resistance.

To understand the role of IR and GLUT4 in glucose homeostasis, mice were engineered to destroy the function of these genes. Genetic ablation of IR in all tissues results in lethality at 4–5 days after birth due to severe diabetic ketoacidosis (11). When GLUT4 is “knocked out” of all tissues (GLUT4-null), mice are growth-retarded, with markedly reduced fat mass, cardiomegaly, and shortened lifespan but no diabetes (12). In contrast, at least 50% of heterozygous GLUT4-null mice developed diabetes by 6 months of age (13). Hence, to distinguish the role of the insulin receptor and GLUT4 in adipose tissue and muscle in glucose homeostasis, diabetes, and adiposity, tissue-specific knock-out mice were made using *Cre/loxP* gene targeting (14). These mice challenge long held concepts about the control of glucose homeostasis (Fig. 1).

Muscle-specific GLUT4^{-/-} Mouse

Muscle-specific GLUT4 knock-out mice (muscle-G4KO) were made by breeding mice carrying the GLUT4 gene with exon 10 flanked by *loxP* sites to mice carrying a transgene encoding the Cre recombinase enzyme under the control of the muscle creatine kinase promoter/enhancer (15). GLUT4 protein levels were reduced about 95% in all skeletal muscles and heart. GLUT1 protein levels were normal in skeletal muscle but were increased by 1.5–2-fold in hearts of muscle-G4KO mice. An even greater induction of cardiac GLUT1 expression was seen in GLUT4-null mice (12) and in mice with cardiac-specific GLUT4 knock-out (cardiac-G4KO) (16).

In contrast to GLUT4-null mice (12), muscle-G4KO mice have normal body weight and fat pad weight at least until 6 months of age. Skeletal muscle mass is also normal. Heart weight is increased, consistent with GLUT4-null mice (12) and cardiac-G4KO mice (16). Lifespan is normal in muscle-G4KO mice in contrast to the shortened lifespan in GLUT4-null mice.

Ex vivo, basal, insulin-stimulated, and contraction-stimulated glucose transport are markedly reduced in both slow twitch, oxidative and fast twitch, glycolytic muscles of muscle-G4KO mice. These mice have hyperglycemia, glucose intolerance, and insulin resistance as early as 8 weeks of age, which persists for up to 1 year of age (15). A subset of mice have frank diabetes with severe insulin resistance. Muscle-G4KO mice do not develop dyslipidemia or other aspects of the metabolic syndrome, in contrast to mice with muscle-specific insulin receptor knock-out (muscle-IRKO), also MIRKO (17)) (see below).

Insulin-stimulated glucose transport in muscle *in vivo* is markedly reduced in muscle-G4KO mice (18). Surprisingly, insulin-stimulated glucose transport in adipose tissue and suppression of hepatic glucose production by insulin are also impaired. The effects in adipose tissue and liver appear to be at least partly due to glucose

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¹ The abbreviations used are: IR, insulin receptor; GLUT, glucose transporter; G4KO, GLUT4 knock-out; IRKO, insulin receptor knock-out; IGF, insulin-like growth factor; GTG, gold thioglucose.

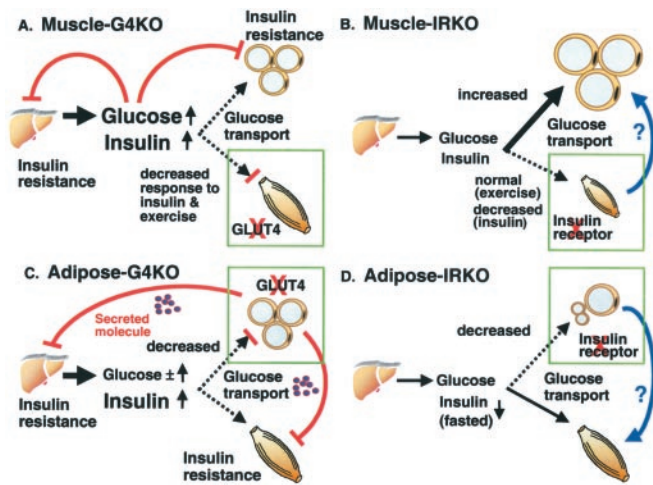


FIG. 1. Changes in glucose homeostasis and adiposity with muscle-specific or adipose-specific ablation of GLUT4 or IR. *A*, muscle-G4KO mouse. Ablation of GLUT4 from muscle (green box) decreases both insulin- and exercise-induced glucose uptake in muscle resulting in hyperglycemia, hyperinsulinemia, and secondary insulin resistance in the liver and adipose tissue. Insulin resistance in the liver and adipose tissue may be caused, at least partly, by glucose toxicity (red curves) (see text). *B*, muscle-IRKO (or MIRKO) mouse. Ablation of IR in muscle (green box) decreases muscle mass but does not change plasma glucose or insulin levels or glucose tolerance. Contraction-stimulated glucose uptake remains normal. Increased glucose uptake into adipose tissue increases adipose mass, serum triglycerides, and free fatty acids. Whether muscle releases a factor that directly acts on adipose tissue is unknown (blue curve). *C*, adipose-G4KO mouse. Ablation of GLUT4 in adipose tissue (green box) does not alter adipose mass, but results in insulin resistance in liver and muscle and systemic hyperinsulinemia. This is most likely due to altered secretion of an unknown molecule(s) from adipose tissue (red curves). Blood glucose is increased in some of the adipose-G4KO mice (symbol \pm). *D*, adipose-IRKO (or FIRKO) mouse. In contrast to the adipose-G4KO mouse, ablation of IR in adipose tissue (green box) decreases adipose mass, unmasks adipocyte heterogeneity, lowers fasting insulin levels, and may increase energy expenditure. This may, in part, be driven by changes in adipocyte-secreted molecules (blue arrow). Red, insulin resistance; blue, insulin action or sensitivity.

toxicity because phloridzin treatment, which normalizes blood glucose in diabetic animals by increasing glucose excretion by the kidney, normalized insulin action in adipose tissue and liver of muscle-G4KO mice (18). In muscle-G4KO and GLUT4-null mice, glucose uptake by the liver may partially compensate for impaired glucose uptake in muscle and other tissues (12, 15).

Why Is the Defect in Glucose Homeostasis in Muscle-G4KO Mice More Severe than in the Homozygous, Whole-body GLUT4-null Mice?—This may reflect differences in the developmental stage at which GLUT4 is deleted or in genetic background. Alternatively, the absence of GLUT4 from discrete regions of the brain, kidney, and other tissues may modify the phenotype in GLUT4-null mice. Additionally, the negative nutritional balance in GLUT4-null mice may prevent the emergence of diabetic features.

Heterozygous GLUT4^{+/-}-null mice become more diabetic than GLUT4-null mice (13). Moreover, diabetes is averted when GLUT4 expression is restored specifically in muscle of GLUT4^{+/-} mice (19), confirming the importance of GLUT4 in muscle to maintain glucose homeostasis. Transgenic overexpression of GLUT1 in skeletal muscle also increases basal glucose uptake and muscle glycogen synthesis but insulin-mediated glucose uptake is impaired (20, 21).

Effects of GLUT4 Ablation on the Heart—Muscle-G4KO, cardiac-G4KO, and GLUT4-null mice all develop cardiac hypertrophy. GLUT4-null homozygous (22) and heterozygous (13) mice show cardiac dysfunction under normal conditions. In contrast, cardiac-G4KO mice have normal contractile function under basal conditions (16) but decreased recovery after hypoxia (23). The increase in GLUT1 expression in the heart of these models clearly cannot compensate for the absence of GLUT4 suggesting that GLUT4 is essential for normal cardiac function at least under some conditions. Furthermore, cardiac-G4KO mice are normal metabolically, thus eliminating the possibility that hyperinsulinemia or other metabolic abnormalities play a major etiologic role in the cardiac hypertrophy. In GLUT4-null mice, however, metabolic abnormalities including alterations in cardiac substrate availability may contribute to impaired cardiac function.

Muscle-specific Insulin Receptor^{-/-} Mouse

Muscle-IRKO (also MIRKO (17)), created by breeding mice with exon 4 of the insulin receptor flanked by *loxP* sites to the muscle creatine kinase-Cre mice, exhibit a muscle-specific >95% reduction in insulin receptor content and early insulin signaling events (17). Insulin-stimulated glucose uptake and activation of glycogen synthase in muscle *in vivo* and *in vitro* are severely impaired. Despite this, muscle glycogen content is relatively normal or only mildly decreased. Unexpectedly, muscle-IRKO mice show no alteration in glucose tolerance in the awake state, and they maintain euglycemia for at least the first 11 months of life (17). Moreover, plasma insulin concentrations and the blood glucose lowering effect of exogenous insulin are normal. These data sharply contrast with the insulin resistance and glucose intolerance in muscle-G4KO (above) and adipose-G4KO mice (below). Euglycemic clamp studies reveal insulin resistance in muscle-IRKO mice (24), but this is not evident under ambient conditions (17).

In contrast to muscle-G4KO mice, muscle-IRKO mice have features of “the metabolic syndrome” including increased fat mass, serum triglycerides, and serum free fatty acids (Table I) (17). Glucose uptake in adipose tissue *in vivo* is increased 3-fold in muscle-IRKO mice whereas glucose uptake in adipocytes is normal *in vitro* (24). This *in vivo* repartitioning of glucose from muscle to adipose tissue suggests that impairment of insulin signaling in muscle, but not impairment of glucose uptake *per se*, can lead to altered adipose insulin sensitivity, increased adiposity, and abnormal plasma lipid profiles. This phenomenon illustrates that tissue “cross-talk” occurs when insulin action is impaired in one insulin target tissue. Interestingly, muscle-IRKO mice and cardiac-specific IRKO mice (25) have decreased cardiac size in contrast to the cardiac hypertrophy when GLUT4 is absent from the heart.

The data suggest that insulin signaling through its cognate receptor in muscle is not essential for the maintenance of glucose disposal in mice (17). The glucose intolerance in muscle-G4KO mice (15), but not in muscle-IRKO mice, suggests that insulin-independent glucose uptake contributes importantly to the maintenance of glucose homeostasis. Unlike mice with muscle-G4KO, mice with muscle-IRKO retain normal basal and contraction-stimulated glucose transport (26). Indeed, muscle-IRKO mice display normal exercise-stimulated glucose uptake and a normal synergistic action of exercise plus insulin on muscle glucose uptake. In contrast, contraction-induced glucose uptake is severely impaired in muscle-G4KO mice (15). Therefore, contraction of postural muscles and other forms of physical activity that elicit non-insulin-dependent glucose uptake could contribute to the maintenance of glucose tolerance in muscle-IRKO mice.

In addition, signaling through the insulin-like growth factor-1 (IGF-1) receptor in muscle may become important when IR is absent. Interference with both IR and IGF-1 receptor signaling with a dominant-negative IGF-1 receptor expressed in muscle leads to insulin resistance and type 2 diabetes (27), and deletion of the IGF-1 gene leads to severe muscle insulin resistance (28).

Adipocyte-specific GLUT4^{-/-} Mouse

Adipose-G4KO mice were made by crossing mice carrying the “floxed” GLUT4 allele with transgenic mice expressing Cre recombinase driven by the adipose-specific fatty acid binding protein (aP2) promoter/enhancer (29). GLUT4 protein levels were reduced by 70–99% in both brown and white adipose tissue whereas GLUT4 levels were preserved in skeletal muscle and heart. In adipocytes from adipose-G4KO mice, basal glucose uptake tends to be reduced, and insulin-stimulated glucose uptake is markedly blunted. Unlike the global GLUT4-null mice (12), reduction of GLUT4 selectively in adipose tissue does not result in growth retardation or decreased adipose mass or adipocyte size (29). The latter suggests that the small amount of glucose transported by GLUT1 in adipocytes may be adequate for the formation of glycerol 3-phosphate required for triglyceride synthesis. Heart weight is also normal in adipose-G4KO mice in contrast to the cardiomegaly observed in GLUT4-null mice and in cardiac-specific (16) and muscle-specific (15) G4KO mice.

Although white adipose tissue accounts for less than 10% of whole-body glucose uptake (30), adipose-G4KO mice have insulin resistance and glucose intolerance, and a subset of these mice

TABLE I
Comparison of muscle-G4KO, muscle-IRKO, adipose-G4KO, and adipose-IRKO mice

N/D, not determined; G4, GLUT4; IR, insulin receptor; TG, triglycerides; GTG, gold thioglucose injection to induce hyperphagia and obesity; ITT, insulin tolerance test. "Clamp" refers to euglycemic-hyperinsulinemic clamp.

	Muscle-G4KO	Muscle-IRKO	Adipose-G4KO	Adipose-IRKO
Growth	Normal	Normal	Normal	Normal
Adiposity	Normal	Increased	Normal	Decreased
Muscle mass	Normal	Decreased	Normal	Normal
Heart mass	Hypertrophy	Decreased	Normal	Normal
Metabolic parameters				
Glucose	Increased	Normal	Normal/increased	Normal/lower (after GTG)
Lactate	Decreased	Normal	Normal/decreased	Normal
FFA	Normal	Increased	Normal	Normal
TG	Normal	Increased	Normal	Decreased
Insulin	Increased	Normal/slight increase	Increased	Decreased (fasting)
Leptin (per adipose mass)	Normal	N/D	Normal	Increased
Adiponectin	Normal	N/D	Normal	Increased
TNF- α	N/D	N/D	Normal	N/D
Glucose metabolism				
Whole-body				
Glucose tolerance	Impaired	Normal (awake) ^a	Impaired	No aging- or obesity-induced glucose intolerance
Insulin sensitivity	Impaired	Normal (ITT)/decreased (clamp)	Impaired	No aging- or obesity-induced insulin resistance
Individual tissues				
Glucose uptake				
Muscle (insulin)	Decreased	Decreased	Decreased (<i>in vivo</i>)/normal (<i>in vitro</i>)	N/D
Muscle (contraction)	Decreased	Normal	N/D	N/D
Adipose tissue (insulin)	Decreased (clamp)	Normal (<i>in vitro</i>)/increased (clamp)	Decreased	Decreased
Insulin-induced suppression of hepatic glucose production	Decreased	Normal	Decreased	N/D

^a When anesthetized, glucose tolerance was slightly impaired (26).

develops extreme insulin resistance and diabetes. The range in severity of the phenotype is expected when studying outbred strains with a mixture of genetic modifiers. Hyperinsulinemic/euglycemic clamp studies reveal an ~50% reduction in whole-body glucose uptake in adipose-G4KO mice. As expected, insulin-stimulated 2-deoxyglucose uptake into white and brown adipose *in vivo* is markedly reduced. However, unexpectedly, glucose uptake into skeletal muscle *in vivo* is also impaired despite preserved GLUT4 expression in muscle. As basal and insulin-stimulated glucose uptake are normal in muscle from these mice *ex vivo*, it appears that this defect is secondary to the *in vivo* milieu. Furthermore, in addition to the insulin resistance in muscle, the insulin-induced suppression of hepatic glucose production is impaired in adipose-G4KO mice. Insulin-stimulated activation of phosphoinositide 3-kinase is impaired in muscle and liver of adipose-G4KO mice *in vivo* (29) and could contribute to the defective insulin responses in these tissues.

These data suggest that reduction of insulin-stimulated glucose transport in adipocytes secondarily induces insulin resistance in other insulin target tissues. Adipocytes can indirectly regulate insulin action and substrate metabolism in muscle and liver and insulin secretion from pancreatic β -cells by altered release of free fatty acids, leptin, tumor necrosis factor- α , resistin, and adiponectin (31, 32). However, serum levels of these molecules are not altered in adipose-G4KO mice (Table I) (29).² Insulin resistance also occurs by increasing lipid depots in muscle and liver, but these were not increased in adipose-G4KO mice. Thus, the insulin resistance in muscle and liver of adipose-G4KO mice is likely to be caused by altered secretion of an as yet unidentified adipocyte-derived molecule that affects insulin action in other tissues (Fig. 1). Additional evidence for a "systemic impact" of adipose-GLUT4 is the fact that adipose-specific overexpression of GLUT4 results in enhanced glucose disposal and insulin sensitivity *in vivo* (33).

Adipocyte-specific Insulin Receptor^{-/-} Mouse

Adipocyte-specific insulin receptor knock-out (adipose-IRKO, also FIRKO (34)) mice were generated by breeding insulin receptor-floxed mice (17) with transgenic mice that express Cre recombinase driven by the aP2 promoter/enhancer. Whereas basal glucose uptake in adipocytes from adipose-IRKO mice is unchanged, insulin-stimulated

glucose uptake is reduced by ~90% (34). Insulin also failed to stimulate glucose metabolism or suppress lipolysis in these adipocytes. Growth is normal in adipose-IRKO mice up to 4 weeks of age. By 8 weeks, however, these mice gained less weight than controls. In contrast to adipose-G4KO mice that have normal adipose mass, adipose-IRKO mice have reduced white adipose and brown adipose tissue mass and whole-body triglyceride content (34).

Blood glucose concentrations were not altered in adipose-IRKO mice at 2–8 months, but fasting plasma insulin concentrations were lower suggesting increased insulin sensitivity (Table I). Serum triglyceride levels were also reduced. Glucose and insulin tolerance tests were normal at 2 and 10 months of age, whereas control mice showed age-related glucose intolerance and insulin resistance. Thus, adipose-IRKO mice are protected against age-related glucose intolerance as well as obesity.

Serum adiponectin concentration was increased in adipose-IRKO mice. Furthermore, plasma leptin levels expressed per mg of fat pad were ~3-fold elevated, and the linear relationship between leptin levels and body weight seen in normal mice was lost. Leptin protein levels were normal in adipose tissue of adipose-IRKO mice. Thus, the absence of IR in adipose tissue alters leptin and adiponectin synthesis, secretion, or clearance.

Adipose-IRKO Mice Are Resistant to Obesity—Mice were injected with gold thioglucose (GTG), which ablates glucose-sensing neurons in the ventromedial hypothalamus. GTG treatment increased food intake in both adipose-IRKO and control mice (34), and as previously observed, control mice treated with GTG became obese. Despite hyperphagia, adipose-IRKO mice did not develop obesity, diabetes, or steatosis in contrast to GTG-injected controls. Thus, adipose-IRKO protects against GTG-induced, as well as age-related, obesity and obesity-related diabetes, and insulin resistance. The protection from obesity could be explained by the lack of the lipogenic and anti-lipolytic effects of insulin in adipocytes. However, it is also likely that energy expenditure is enhanced, because adipose IRKO mice are lean despite hyperphagia.

The reduced adipose mass in adipose-IRKO mice is not due to fewer adipocytes. Histology showed polarization of adipocytes into small and large in contrast to a normal distribution in control mice (34). IR expression in both large and small adipocytes of adipose-IRKO mice is reduced by 85–99%, indicating that the heterogeneity is not due to differences in efficiency of gene recombination. However, expression levels of some proteins, *e.g.* fatty-acid synthase

² B. B. Kahn, unpublished results.

and the adipogenic transcription factors SREBP-1 and C/EBP α , are different in small and large adipocytes, and this may contribute to the heterogeneity.

In contrast to adipose-IRKO mice, brown adipocyte-specific insulin receptor knock-out (BAT-IRKO) mice exhibit an age-dependent loss of brown adipose tissue that is associated with impaired glucose tolerance without insulin resistance (35). This appears to be primarily due to loss of β -cell mass and defective insulin secretion. Thus, absence of IR in white adipose tissue has a protective effect over the impaired glucose homeostasis resulting from the absence of IR in brown adipose tissue alone. In addition, brown adipose tissue may affect pancreatic β -cell mass and function.

Adipose-IRKO Mice Have Enhanced Longevity—Surprisingly, mean lifespan in adipose-IRKO mice is increased by 134 days (18%) (36) in contrast to the shortened lifespan in GLUT4-null mice. Calorie restriction in many species is associated with increased longevity, but it has not been clear whether this is due to decreased food intake or the resultant leanness. Because adipose-IRKO mice do not eat less, the effect of calorie restriction on longevity is likely to be due to reduced adipose mass *per se*. Combined with data showing that decreased signaling through an insulin-like pathway increases longevity in *Caenorhabditis elegans* and *Drosophila melanogaster* (37), these new data imply that selective reduction of insulin signaling in certain metabolically important organs such as adipose tissue could result in extended longevity (36).

Conclusions

Tissue-conditional knock-out mice advance our understanding of the mechanisms underlying insulin resistance. Marked reduction of GLUT4 in muscle or adipose tissue causes insulin resistance in other insulin target tissues secondarily and increases the risk for diabetes. Hence, there is cross-talk among insulin target tissues. This may have implications for the genetics of type 2 diabetes because a single genetic defect in one insulin target tissue can result in insulin resistance in other tissues, leading to type 2 diabetes. This may explain, at least in part, the surprising finding that reduction of GLUT4 in adipose tissue causes a similar degree of insulin resistance as reduction of GLUT4 in muscle. In contrast, ablation of IR in adipose tissue or muscle has only a subtle direct impact on glucose homeostasis, possibly due to compensation by IGF-1 receptor signaling and by insulin-independent stimulation of glucose transport by contraction. However, IR in both muscle and adipose tissue is critical for the regulation of adiposity.

These models demonstrate a central role for adipose tissue as an endocrine organ in the maintenance of insulin sensitivity and energy balance. The importance of GLUT4 in adipose tissue is particularly relevant in light of selective reduction of adipocyte-GLUT4 expression in obesity and diabetes (4, 5). Data from adipose-G4KO mice indicate that this could contribute to the pathogenesis of insulin resistance in obesity and diabetes, probably by altering the release of novel adipocyte-secreted molecules. Thus, studies using tissue-conditional knock-outs reveal the complexity of glucose homeostasis and suggest that "inter-tissue communication" can modify the impact of specific genetic defects in individual tissues on the pathogenesis of obesity and type 2 diabetes.

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